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(57) Abstract

Ebola virus vaccines comprising nucleic acid molecules encoding Ebola viral proteins are provided. In one embodiment, the nucleic acid molecule encodes the transmembrane form of the viral glycoprotein (GP). In another embodiment, the nucleic acid molecule encodes the secreted form of the viral glycoprotein (sGP). In yet another embodiment, the nucleic acid molecule encodes the viral nucleoprotein (NP). Methods for immunizing a subject against disease caused by infection with Ebola virus are also provided.

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IMMUNIZATION FOR EBOLA VIRUS INFECTION

FIELD OF THE INVENTION

The present invention relates generally to viral vaccines and, more particularly, to Ebola virus vaccines and methods of protecting against disease caused by infection with Ebola virus.

BACKGROUND OF THE INVENTION

The Ebola viruses, and the genetically-related Marburg virus, are filoviruses associated with outbreaks of highly lethal hemorrhagic fever in humans and primates in North America, Europe, and Africa. Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Peters, C.J. et al , *Semin. Virol.* 5:147-154 (1994). Ebola viruses are negative-stranded RNA viruses comprised of four subtypes, including those described in the Zaire, Sudan, Reston, and Ivory Coast episodes. Sanchez, A. et al., *PNAS (USA)* 93:3602-3607 (1996). Although several subtypes have been defined, the genetic organization of these viruses is similar, each containing seven linearly arrayed genes. Among the viral proteins, the envelope glycoprotein exists in two alternative forms, a 50-70 kilodalton (kDa) secreted protein of unknown function encoded by the viral genome and a 130 kDa transmembrane glycoprotein generated by RNA editing that mediates viral entry. Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Sanchez, A. et al., *PNAS (USA)* 93:3602-3607 (1996). Other structural gene products include the nucleoprotein (NP), matrix proteins VP24 and VP40, presumed nonstructural proteins VP30 and VP35, and the viral polymerase (reviewed in Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996)). Although spontaneous variation of its RNA sequence does occur in nature, there appears to be less nucleotide polymorphism within Ebola subtypes than among other RNA viruses (Sanchez, A. et al., *PNAS (USA)* 93:3602-3607 (1996)), suggesting that immunization may be useful in protecting against this disease. Previous attempts to elicit protective immune responses against Ebola virus using traditional active and passive immunization approaches have, however, not succeeded. Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Clegg, J.C.S.

et al., *New Generation Vaccines.* (eds., Levine, M.M., Woodrow, G.C., Kaper, J.B.& Cobon, G.S.) 749-765 (New York, NY, Marcel Dekker, Inc. 1997); Jahrling, P.B. et al., *Arch. Virol. Suppl.* 11:135-140 (1996).

It would thus be desirable to provide a vaccine to protect against disease caused by infection with Ebola virus. It would further be desirable to provide methods of making and using said vaccine.

SUMMARY OF THE INVENTION

Ebola virus vaccines comprising nucleic acid molecules encoding Ebola viral proteins are provided. In one embodiment, the nucleic acid molecule encodes the transmembrane form of the viral glycoprotein (GP). In another embodiment, the nucleic acid molecule encodes the secreted form of the viral glycoprotein (sGP). In yet another embodiment, the nucleic acid molecule encodes the viral nucleoprotein (NP).

The present invention also provides methods for immunizing a subject against disease caused by infection with Ebola virus comprising administering to the subject an immunoeffective amount of an Ebola virus vaccine. Administration can be by any of the routes normally used for gene therapy. In a preferred method, the Ebola virus vaccine is administered by intramuscular injection. The genetic immunization methods of the present invention provide protective immunity against disease caused by infection with Ebola virus.

Additional objects, advantages, and features of the present invention will become apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings.

Figures 1A and 1B are photographs showing expression of Ebola virus gene products in eukaryotic plasmid expression vectors.

Figure 1A. Expression vectors encoding the indicated viral gene products under regulation of the CMV immediate-early region 1 enhancer and promoter were prepared and transfected into 293 cells as previously described. Manthorpe, M. et al. *Hum. Gene. Ther.* 4:419-431 (1993); Sambrook, J., Fritch, E.F., & Maniatis, T. Cold Spring Harbor, N.Y. Cold Spring Laboratory Harbor Press, 1994. Cell extracts were prepared and analyzed by Western blot analysis for NP (left) or GP (right) using

relevant rabbit antisera and a secondary antibody, horseradish peroxidase conjugated donkey anti-rabbit IgG of a dilution of 1:5,000. Incubation with primary antibody was for 30 minutes at room temperature, and for 30 minutes at room temperature with secondary antibody. Immunocomplexes were then detected by chemiluminescence using super signal substrate reagents (Pierce) according to manufacturer's instructions.

Figure 1B. Generation of antibody response in mice immunized with the indicated vectors and analyzed by Western blot for NP, GP, and sGP as shown. Antisera from mice were tested at a dilution of 1:500 (NP), 1:50 (GP), or 1:50 (sGP), respectively, and developed with a secondary antibody (sheep anti-mouse, 1:5,000, Amersham Life Science) and chemiluminescence as in Figure 1A. The control vector used for immunization represents the expression vector plasmid with no insert. Manthorpe, M. et al., *Hum. Gene. Ther.* 4:419-431 (1993).

Figures 2A-2D are graphs showing the immune responses to NP and GP after genetic immunization in mice.

Figure 2A. Splenic lymphocytes from vector or NP-plasmid immunized mice were isolated approximately 6 weeks after the initial immunization and sensitized *in vitro* for 5 days with 10 U/ml hIL-2. Renca-NP cells sensitized splenocytes from vector-immunized or pCMV-NP immunized mice were used to detect CTL activity at the indicated effector:target ratios on Renca or Renca-NP cells (left, middle) or with allogeneic effector cells with Renca-NP to show that they are susceptible to lysis (right). Allogeneic effector cells were generated by incubating cells derived from mice with a C57Bl/6 background ($5x10^6$/ml) with irradiated Balb/c spleen cells ($5x10^6$/ml) in the presence of IL-2 (20 U/ml) for five days. The chromium release CTL assay with Renca-NP cells was performed in triplicate as previously described. Ohno, T. et al., *Gene. Ther.* 4:361-366 (1997).

Figure 2B. Balb/C female mice were immunized with the sGP plasmid expression vector and analyzed for their ability to lyse the syngeneic Renca cell line stably expressing GP. Isolation of stable transfectants, confirmation of expression, and CTL assay were performed as described (see, Specific Example, II. Methods). Renca-GP or sGP sensitized splenocytes from pCMV-GP or pCMV-sGP immunized mice were used to determine the specific killing of ^{51}chromium labeled Renca-GP cells at the indicated E/T ratios.

Figure 2C. Mice immunized with GP were analyzed for their ability to lyse a syngeneic CT26 cell stably expressing GP or CT26 vector control transduced line at the indicated E/T ratios.

Figure 2D. Cellular proliferative response in the indicated immunized mice. T cells, enriched or depleted (see, Specific Example, II. Methods), were incubated at 10^5 cells/ml with sGP condition media (25%). Background was determined with cells incubated in media from control transfected 293 cells and subtracted from proliferation seen in sGP-containing supernatants.

Figures 3A-3C are graphs showing immunization with sGP or GP expression plasmids induces T cell responses to sGP in guinea pigs.

Figures 3A-3C. Cell-mediated immunity in guinea pigs was analyzed by performing assays to detect cell proliferation to control or GP antigen (A) or T-cell growth factor production in response to the indicated antigens. The culture supernatants containing these antigens were prepared as previously described (Bottomly, K. et al., Measurement of human and murine interleukin 2 and interleukin 4. in *Current Protocols in Immunology.* (eds., Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M.& Strober, W.) 6.3.1-6.3.12 (New York, John Wiley & Sons, Inc. 1992); Arai, H. et al., *Nat. Med.* 3:843-848 (1997)), and included at a final concentration of 10% (volume/volume). In A, cell numbers refer to the concentration of spleen cells per ml in the ^{3}H-thymidine proliferation assay. In B, supernatants from A, harvested at the time of the peak proliferative response to sGP, were incubated with primary guinea pig T cells maintained in 200 U/ml of human IL-2. The percent maximal response refers to the magnitude of stimulation in response to the indicated stimuli relative to supernatants from 24 hour concanaval (in A-stimulated cells (2 μg/ml)). The requirement of T lymphocytes in guinea pig spleen cells for the proliferative response to sGP, performed as described in Specific Example, II. Methods, is shown (C).

Figures 4A-4F are photographs showing the immunohistochemical analysis of Ebola virus antigens in liver, lung, and spleen from representative protected (GP-animal 3) or infected (vector-animal 2) guinea pigs.

Figures 4A-4F. Magnification: liver, 40x; lung, 20x; spleen, 20x.

Figure 5 is a schematic of the plasmid pVR 1012-GP(IC) (Ivory Coast strain of GP, SEQ ID NO: 1).

Figure 6 is a schematic of the plasmid pVR 1012-GP(S) (Sudan strain of GP, see SEQ ID NO: 2).

Figure 7 is a schematic of the plasmid pVR 1012-GP(Z) (Zaire strain of GP, see SEQ ID NO: 3).

Figure 8 is a schematic of the plasmid pVR 1012-sGP(Z) (Zaire strain of sGP, see SEQ ID NO: 4).

Figure 9 is a schematic of the plasmid pVR 1012-NP.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Ebola virus vaccines are provided comprising a nucleic acid molecule encoding an Ebola viral protein operatively-linked to a control sequence in a pharmaceutically acceptable carrier. In one embodiment, the nucleic acid molecule encodes the transmembrane form of the viral glycoprotein (GP). In another embodiment, the nucleic acid molecule encodes the secreted form of the viral glycoprotein (sGP). In yet another embodiment, the nucleic acid molecule encodes the viral nucleoprotein (NP).

The present invention further includes vaccines comprising nucleic acid molecules encoding Ebola viral proteins other than GP, sGP, and NP, *e.g.*, other structural gene products which elicit protective immunity from disease caused by infection with Ebola virus. The nucleic acid molecules of the vaccines of the present invention encode structural gene products of any Ebola viral strain including the Zaire, Sudan, Ivory Coast and Reston strains. Nucleic acid molecules encoding structural gene products of the genetically-related Marburg virus strains may also be employed. Moreover, the nucleic acid molecules of the present invention may be modified, *e.g.*, the nucleic acid molecules set forth herein may be mutated, as long as the modified expressed protein elicits protective immunity from disease caused by infection with Ebola virus. For example, the nucleic acid molecule may be mutated so that the expressed protein is less toxic to cells. The present invention also includes vaccines comprising a combination of nucleic acid molecules. For example, and without limitation, nucleic acid molecules encoding GP, sGP and NP of the Zaire, Sudan and Ivory Coast Ebola strains may be combined in any combination, in one vaccine composition.

The present invention also provides methods for immunizing a subject against disease caused by infection with Ebola virus comprising administering to the subject an immunoeffective amount of an Ebola virus vaccine. Methods of making and using Ebola virus vaccines are also provided by the present invention including the preparation of pharmaceutical compositions.

As referred to herein, the term "encoding" is intended to mean that the subject nucleic acid may be transcribed in a cell, *e.g.*, when the subject nucleic acid is linked to appropriate control sequences such as a promoter in a suitable vector (*e.g.*, an expression vector) and the vector is introduced into a cell. The nucleic acid molecules of the present invention may be DNA molecules, cDNA molecules or RNA molecules, and are preferably cDNA molecules. The term "operatively-linked" as used herein refers to functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence. Expression control sequences are known to those skilled in the art (see, *e.g.*, Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990)). Vectors which contain both a promoter and a cloning site to which an inserted piece of nucleic acid is operatively-linked to the promoter, are well known in the art and are generally referred to herein as "expression vectors" or "expression vector plasmids". Preferably, these vectors are capable of transcribing nucleic acid *in vitro* and *in vivo*. A preferred vector is the cytomegalovirus (CMV) expression vector which directs high levels of gene expression in muscle.

Nucleic acid molecules which hybridize under stringent conditions to the nucleic acid molecules described herein are also within the scope of the present invention. As will be appreciated by those skilled in the art, multiple factors are considered in determining the stringency of hybridization including species of nucleic acid, length of nucleic acid probe, T_m (melting temperature), temperature of hybridization and washes, salt concentration in the hybridization and wash buffers, aqueous or formamide hybridization buffer, and length of time for hybridization and for washes. An example of stringent conditions are DNA-DNA hybridization with a probe greater than 200 nucleotides in 5 x SSC, at 65°C in aqueous solution or 42°C in formamide, followed by washing with 0.1 x SSC, at 65°C in aqueous solution. (Other experimental conditions for controlling stringency are described in Maniatis, T. et al., *Molecular Cloning: a Laboratory Manual*, Cold Springs Harbor Laboratory, Cold Springs, N.Y. (1982) at pages 387-389 and Sambrook, J. et al., *Molecular Cloning: a Laboratory Manual*, Second Edition, Volume 2, Cold Springs Harbor Laboratory, Cold Springs, N.Y., at pages 8.46-8.47 (1989)).

It will be appreciated that administration of the vaccines of the present invention can be by any of the routes normally used for gene therapy. In a preferred

method, administration is by intramuscular injection, however, other procedures for transfecting cells may also be employed, such as transfection using calcium phosphate coprecipitation, liposome-mediated transfection, plasmid and viral vector-mediated transfection and DNA protein complex-mediated transfection. Viral vector-mediated transfection includes, without limitation, the use of retroviral, replication-deficient retroviral, adenoviral and adeno-associated viral vectors. Cells transfected by the vaccines in the context of *ex vivo* gene therapy can also be administered.

It will be appreciated that more than one route of administering the vaccines of the present invention may be employed either simultaneously or sequentially (*e.g.*, boosting). In addition, the vaccines of the present invention may be employed in combination with traditional immunization approaches such as employing protein antigens, vaccinia virus and inactivated virus, as vaccines. Thus, in one embodiment, the vaccines of the present invention are administered to a subject (the subject is "primed" with a vaccine of the present invention) and then a traditional vaccine is administered (the subject is "boosted" with a traditional vaccine). In another embodiment, a traditional vaccine is first administered to the subject followed by administration of a vaccine of the present invention. In yet another embodiment, a traditional vaccine and a vaccine of the present invention are co-administered.

Immunogenicity may be significantly improved if the vaccines of the present invention are co-administered with an immunostimulatory agent or adjuvant. Adjuvants enhance immunogenicity but are not necessarily immunogenic themselves. Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Adjuvants may thus be employed to enhance the immunogenicity of the vaccines of the present invention, as well as the immunogenicity of traditional vaccines. Suitable adjuvants are well known to those skilled in the art and include, without limitation, aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octodecyl ester of an amino acid, a muramyl dipeptide, polyphosphazene, a lipoprotein, ISCOM matrix, DC-Chol, DDA, and other adjuvants and bacterial toxins, components and derivatives thereof.

The vaccines of the present invention may also be co-administered with cytokines to further enhance immunogenicity. The cytokines may be administered by methods known to those skilled in the art, *e.g.*, as a nucleic acid molecule in plasmid form or as a protein or fusion protein.

Upon inoculation with a pharmaceutical composition as described herein, the immune system of the host responds to the vaccine by producing antibodies, both secretory and serum, specific for Ebola virus proteins. As a result of the vaccination, the host becomes at least partially or completely immune to Ebola virus infection, or resistant to developing moderate or severe disease caused by Ebola virus infection. Although Ebola virus infection and disease caused thereby are discussed in detail herein, it will be appreciated that the vaccines and methods of the present invention may be employed to immunize a subject against hemorrhagic fever generally, such as that caused by infection by the genetically-related Marburg virus.

Pharmaceutical compositions comprising the nucleic acid molecules encoding Ebola viral proteins described herein, either alone or in combination, and a pharmaceutically acceptable carrier, are also provided by the present invention. As used herein, the phrase "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as those suitable for parenteral administration, such as, for example, by intramuscular, intraarticular (in the joints), intravenous, intradermal, intraperitoneal, and subcutaneous routes. Examples of such formulations include aqueous and non-aqueous, isotonic sterile injection solutions, which contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the vaccine dissolved in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the vaccine, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; (d) suitable emulsions; and (e) polysaccharide polymers such as chitians. The vaccine, alone or in combination with other suitable components, may also be made into aerosol formulations to be administered via inhalation, *e.g.*, to the bronchial passageways. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the vaccine with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a

combination of the vaccine with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the recipient, *e.g.*, the patient. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules or vials and may be prepared by any method known in the art.

Pharmaceutical compositions comprising any of the nucleic acid molecules encoding Ebola viral proteins of the present invention are useful to immunize a subject against disease caused by Ebola virus infection. Thus, this invention further provides methods of immunizing a subject against disease caused by Ebola virus infection, *e.g.*, hemorrhagic fever, comprising administering to the subject an immunoeffective amount of a pharmaceutical composition of the invention. This subject may be an animal, for example a mammal, such as a primate or preferably a human.

The vaccines of the present invention are also suitable for veterinary immunization. The vaccines of the present invention comprising nucleic acid molecules encoding Ebola virus structural gene products from the Reston strain, which is known to infect animals, are particularly useful in such veterinary immunization methods.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, immunogenic and protective. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the immune system of the individual to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and may be monitored on a patient-by-patient basis. However, suitable dosage ranges are readily determinable by one skilled in the art and generally range from about 300 μg to about 4-5 mg. The dosage may also depend, without limitation, on the route of administration, the patient's state of health and weight, and the nature of the formulation.

Methods of immunizing a subject against multiple strains of Ebola virus are further provided herein. The nucleic acid molecules encoding Ebola viral proteins of the present invention may be combined with nucleic acid molecules enooding other viral proteins of other virus strains to achieve protection against multiple strains of

Ebola virus. Typically the vaccines will be in an admixture and administered simultaneously, but may also be administered separately.

In some instances it may be desirable to combine the Ebola virus vaccines of the present invention with vaccines which induce protective responses to other agents, particularly other viruses. For example, the vaccine compositions of the present invention can be administered simultaneously, separately or sequentially with other genetic immunization vaccines such as those for influenza (Ulmer, J.B. et al., *Science* 259:1745-1749 (1993); Raz, E. et al., *PNAS (USA)* 91:9519-9523 (1994)), malaria (Doolan, D.L. et al., *J. Exp. Med.* 183:1739-1746 (1996); Sedegah, M. et al., *PNAS (USA)* 91:9866-9870 (1994)), and tuberculosis (Tascon, R.C. et al., *Nat. Med.* 2:888-892 (1996)).

It will also be appreciated that single or multiple administrations of the vaccine compositions of the present invention may be carried out. For example, subjects who are particularly susceptible to Ebola virus infection may require multiple immunizations to establish and/or maintain protective immune responses. Levels of induced immunity can be monitored by measuring amounts of neutralizing secretory and serum antibodies, and dosages adjusted or vaccinations repeated as necessary to maintain desired levels of protection.

This invention also provides kits comprising the vaccines of the present invention. For example, kits comprising a vaccine and instructions for use are within the scope of this invention.

The vaccines and methods of the present invention evoke a protective immune response and do not lead to immunopotentiation or exacerbated disease. The vaccines lack transmissibility, are genetically stable and induce protective levels of humoral and cell-mediated immunity.

In order to more fully demonstrate the advantages arising from the present invention, the following example is set forth. It is to be understood that the following is by way of example only and is not intended as a limitation on the scope of the invention.

SPECIFIC EXAMPLE

I. RESULTS

Immune response to viral gene products in mice. To characterize immune responses to selected Ebola virus proteins, eukaryotic expression vector plasmids were injected into mice. The cytomegalovirus (CMV) immediate early region 1 enhancer was used to stimulate transcription because it directs high levels of gene

expression in muscle. Manthorpe, M. et al., *Hum. Gene. Ther.* 4:419-431 (1993). cDNAs encoding an abundant structural protein, the major viral nucleocapsid phosphoprotein (NP), the secreted glycoprotein (sGP), or the membrane-associated glycoprotein (GP) were inserted. Alternative forms of GP were chosen because it had been postulated that the transmembrane protein contained a protein sequence motif also found in oncogenic retroviruses that might suppress immune responses. Burkreyev, A.A. et al., *FEBS. Lett.* 323:183-187 (1993); Cianciolo, G.J. et al., *Science* 230:453-455 (1985); Harris, D.T. et al., *J. Immunol.* 138:889-894 (1987); Volchkov, V.E. et al., *FEBS. Lett.* 305:181-184 (1992); Sanchez, A. et al., *Virus. Res.* 29:215-240 (1993). Expression of the relevant proteins was confirmed after transfection of the human renal epithelial cell line, 293, by immunoblotting with antisera to these gene products (Fig. 1A). For NP, the expected full-length 104 kDa protein normally produced by the virus was seen, together with lower molecular weight species likely generated from truncated protein or degradation products described previously. Sanchez, A. et al., *Virology* 170:81-91 (1989). Similarly, expression of sGP and GP revealed a heterogeneous pattern whose sizes correlated with the expected products of cleavage or post-translational carbohydrate modification. Sanchez, A. et al., *PNAS (USA)* 93:3602-3607 (1996).

These plasmids were injected into mice to characterize their ability to induce humoral and cellular immune responses to the relevant viral proteins. Three injections, each with 50 μg of plasmid DNA in saline (100 μl), were performed at two-week intervals in Balb/C female mice (6-8 week old, Charles River). Serum from immunized recipients were examined for antibody responses. An antibody response to the viral NP gene product was readily detectable (Fig. 1B), with titers of $\geq$ 1:16,000 by Western blot analysis. The titer of antibody induced in response to injection with plasmids encoding the viral glycoproteins was lower. After immunization with GP, no antibody was detectable by Western blotting, while immunization with sGP induced an antibody response (Fig. 1B). The more sensitive ELISA (Ksiazek, T.G., *Lab. Anim.* 20:34-46 (1991); Ksiazek, T.G. et al., *J. Clin. Microbiol.* 30:947-950 (1992)) did allow detection of antibodies to both GP and sGP at titers of 1:400 and 1:1,200, respectively. Cytolytic T cell (CTL) responses to these viral proteins were analyzed next. Despite the substantial humoral immune response to NP, minimal CTL activity was detected against syngeneic cells expressing this viral protein (Fig. 2A). In contrast, genetic immunization with sGP, which elicited a weaker antibody response, induced a marked cytolytic T cell response to cells expressing GP (Fig. 2B).

Immunization with the GP plasmid also induced a significant CTL response to GP (Fig. 2C). These data suggested that both the secreted and transmembrane form of the protein could be processed for antigen presentation and the transmembrane form was a target for recognition by these cytolytic T cells. Finally, antigen-specific T cell proliferation to sGP was also observed in GP and sGP but not plasmid control injected mice (Fig. 2D).

The ability of antibodies detected in mouse sera after immunization to neutralize virus was tested in an *in vitro* infection assay. McCormick, J.B. et al., *J. Infect. Dis.* 147:264-267 (1983). In no case was neutralization of infectivity observed, even at titers of 1:10 (data not shown), despite the documented presence of antibody after NP and sGP immunization by Western blot analysis. Infectivity *in vitro* was thus not inhibited by Ebola-specific antibodies.

Immune function and viral challenge in guinea pigs. To determine whether the *in vivo* immune responses could protect against viral infection, virus was adapted to grow in guinea pigs. Though this species is not well-suited to analysis of immune function, infection in adult mice has not been successful. Moreover, infection in guinea pigs, used originally to propagate virus from infected humans, is a well-established animal model for the human disease. Infection gives rise to a syndrome of hemorrhagic fever with levels of virus, organ pathology, and infection of reticuloendothelial and mononuclear cells comparable to humans. Bowen, E.T.W. et al., *Lancet* 1:571-573 (1977).

Two groups of immunized guinea pigs were studied. Animals were injected intramuscularly with the relevant expression vector plasmids, and the response to infection in groups immunized with either sGP, GP, NP, or control plasmids was observed. In the first group, animals were challenged within 2 months after the initial immunization, at which time the antibody titers were high, ranging from 1:1,600 to >1:25,000 (Table 1A). In these animals, nearly complete protection from lethal challenge was observed in GP (6/6), sGP (5/6), and NP (4/4) immunized subjects, in contrast to controls (0/6). In a second group, guinea pigs were challenged four months after the initial immunization (Table 1B). As in the first group, all animals immunized with the control vector succumbed to infection within a week after virus challenge (n=4). In this group, antibody titers were lower, and three of the four guinea pigs immunized with NP succumbed to infection, with the single survivor appearing severely ill after 1 week, in contrast to the prot ctive response with NP at the earlier time point after immunization in Group I. More effective protection was

seen in animals immunized with vector expressing GP, protection was noted in four of five animals challenged, with one survivor appearing weak but surviving the viral challenge. Similarly, three of the five animals immunized with sGP showed no ill effects following viral challenge. Protection in this group again correlated with the ability to sustain an effective immune response to GP or sGP. Together, all guinea pigs immunized with vectors expressing GP or sGP which had titers greater than 1:5,120 were resistant to infection (11/11) compared to 0/10 controls (p=0, by Fisher's exact test). Twelve of fourteen animals with antibody titers ≥2,560 survived viral challenge compared to controls (p=.00003, by Fisher's exact test). Similar to immunized mice, guinea pigs injected with GP or sGP plasmids were able to generate cell-mediated immune responses to the viral glycoprotein in addition to the antibody response. These responses were antigen-specific and T cell-dependent, as detected in sGP antigen-dependent spleen cell proliferation and T-cell growth factor assays (Fig. 3A-C). Thus, the ability to generate and sustain significant cellular immune responses *in vivo* correlated with protection from infection. Though antibody titer correlated with protection, cell-mediated immunity appeared necessary for protection since passive transfer of antibody to GP does not confer protection (Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Jahrling, P.B. et al., *Arch. Virol. Suppl.* 11:135-140 (1996)) and antisera from protected guinea pigs did not inhibit virus replication *in vivo* (n=3) or at a 1:10 dilution *in vitro* (data not shown). Since the Hartley guinea pig to which the virus has been adapted for growth is outbred, cellular adoptive transfer studies could not be performed.

TABLE 1 - Group I

Plasmid	Subject	ELISA(Pre)	ELISA(Post)	Viral Ag	Survival
GP	1	>1:25,600	1:12,800	-	Yes
GP	2	>1:25,600	1:25,600	-	Yes
GP	3	>1:25,600	1:25,600	-	Yes
GP	4	1:25,600	1:6,400	-	Yes
GP	5	1:25,600	1:12,800	-	Yes
GP	6	1:25,600	1:25,600	-	Yes
SGP	1	1:12,800	1:25,600	-	Yes
SGP	2	1:6,400	1:25,600	-	Yes
SGP	3	1:6,400	1:25,600	-	Yes

SGP	4	1:25,600	1:25,600	-	Yes
SGP	5	>1:25,600	1:12,800	-	Yes
SGP	6	1:1,600	Negative	+	No
NP	1	1:12,800	>1:25,600	-	Yes
NP	2	>1:25,600	1:25,600	-	Yes
NP	3	1:12,800	1:12,800	-	Yes
NP	4	1:25,600	1:25,600	-	Yes
Vector alone	1	Negative	Negative	+	No
Vector alone	2	Negative	n.d.	+	No
Vector alone	3	Negative	Negative	+	No
Vector alone	4	Negative	Negative	+	No
Vector alone	5	Negative	n.d.	+	No
Vector alone	6	Negative	n.d.	+	No

Guinea pigs were immunized on days 1, 14, 28, 42, and challenged on day 62.

TABLE 1 - Group II

Plasmid	Subject	ELISA(Pre)	ELISA(Post)	Viral Ag	Survival
GP	1	1:2,560	n.d.	+/f	No
GP	2	1:5,120	1:10,240	-	Yes
GP	3	1:10,240	1:10,240	-	Yes
GP	4	1:1,280	n.d.	+/f	No
GP	5	1:5,120	1:20,480	-	Yes (ill)
SGP	1	1:2,560	n.d.	+	No
SGP	2	1:10,240	1:5,120	+/f	Yes
SGP	3	1:10,240	1:81,920	-	Yes
SGP	4	1:2,560	1:5,120	-	Yes
SGP	5	1:640	n.d.	+	No
NP	1	n.d.	n.d.	+	No
NP	2	n.d.	n.d.	+	No
NP	3	n.d.	n.d.	+	No
NP	4	n.d.	Negative	+	Yes (ill)
Vector alone	1	Negative	n.d.	+	No
Vector alone	2	Negative	n.d.	+	No
Vector alone	3	Negative	n.d.	+	No
Vector alone	4	Negative	n.d.	+	No

Guinea pigs were immunized on days 1, 14, 42, and 112 and challenged on day 122.
n.d.=not done. Viral ag denotes presence of virus determined by immunohistochemistry (30) performed on spleen, liver, lung, kidney, and heart tissues; "+" = widespread systemic involvement of the mononuclear phagocyte system and to a lesser extent endothelial and parenchymal cells; "+/f" = focal involvement (seen in the spleen of SGP #2, the liver and spleen of GP #1, and the lung of GP#4) where rare sites of anti-Ebola antibody staining were detected.; "-" = no Ebola virus antigen detected in tissues.
ELISA determinations made prior to viral challenge (Pre) or at least 7 days after (Post) infection, respectively.
The surviving NP immunized animal (4) was found to have significant levels of virus in major organs by immunohistochemistry, and more than 5 logs of virus was detected in the serum and spleen, in contrast to GP and sGP animals where no virus was detected.

Histopathologic analysis of infection in immunized guinea pigs. Pathologic analysis revealed widespread tissue necrosis and dissemination of virus by immunohistochemistry, similar to human disease. Virus load correlated with susceptibility to infection with titers of $\geq 10^5$ in infected animals compared to undetectable levels in immunized survivors. In infected animals, the liver, lung, and spleen showed evidence of significant viral antigen by immunohistochemistry (Fig. 4, Table 1), and both reticuloendothelial and mononuclear phagotic involvement was observed.

Determination of antibody response in animals which survived virus challenge revealed increases in the immune response to viral proteins when initial titers were lower (Table 1). Less consistent increases in antibody titers were observed in the NP immunized animals. These data suggest that Ebola virus infection may stimulate immunity in survivors of a viral challenge when immune responses are not optimal.

II. METHODS

Plasmids. Plasmids containing the GP, sGP, or NP cDNAs (Sanchez, A. et al., *Virus. Res.* 29:215-240 (1993), Genbank) were used to subclone the relevant inserts into CMV expression vectors which utilized the bovine growth hormone polyadenylation sequence. Manthorpe, M. et al., *Hum. Gene. Ther.* 4:419-431 (1993). (see Figures 5-9 and SEQ ID NOS: 1-4). Briefly, for GP, plasmid pGEM-3Zf(-)-GP was digested with EcoR I, treated with the Klenow fragment of E. coli DNA polymerase, and digested with BamH I. The GP fragment was then inserted into the pCMV expression vector plasmid digested with BamH I, Klenow fragment and Bgl II. For sGP, the plasmid pCRH-sGP was digested with EcoR I, treated with Klenow

enzyme, and the resulting fragment inserted into the BamH I/Bgl II CMV plasmid which had been incubated with Klenow fragment, calf intestinal phosphatase (CIP), then phenol chloroform extract. For the NP expression vector, plasmid pSP64-NP2 (Sanchez, A. et al., *Virology* 170:81-91 (1989)) was digested with EcoR I, treated with Klenow enzyme, and digested with BamH I. The NP insert was cloned into CMV treated with BamH I, Klenow enzyme, followed by heat inactivation and Bgl II digestion.

Cell lines and transfectants. For stable transfectants, the relevant cDNAs were inserted into a CMV expression plasmid containing a neomycin resistant gene, pCMV-neo (H. Arai, unpublished data), which was digested with Xba I, and treated with CIP and Klenow enzyme. The EcoR I/ BamH I GP fragment from pGEM-3Zf(-)-GP, the EcoR I sGP fragment from pCRII-SGP, or the EcoR I/BamH I NP fragment from pSP64-NP2 was treated with Klenow enzyme and ligated to this plasmid backbone. These vectors were transfected into Renca or CT26 which was syngeneic to Balb/C mice using calcium phosphate and selected in 0.7 or 1mg/ml G418 for 2-6 weeks. Expression of GP, sGP, or NP from these vectors in Renca or CT26 cells was also confirmed by Western blot analysis (data not shown).

Cell proliferation assay. Spleen cells from male Hartley guinea pigs or Balb/C female mice (8-10 weeks) immunized with the indicated plasmid expression vectors were incubated with sGP or vector control supernatants (25% volume:volume) from transfected 293 cells at the indicated cell concentrations. T cell depletion was performed using the CT5 monoclonal antibody (Tan, B.T.G. et al., *Hybridoma* 4:115-124 (1985)) (Biosource, Camarillo, CA) for guinea pigs or anti-Thy 1.2 antibody in the mouse using immunomagnetic microbeads (Miltenyi Biotec, Inc., Auburn, CA).

Viral challenge in guinea pigs. Animals were immunized by injection of 100 µl (0.5 mg/ml) in each hind leg (two injections at each time point) with the indicated plasmid expression vectors. Animals were challenged by inoculation with a stock of Ebola virus (Zaire, 1976) that had been passaged once in vero E6 cells and serially passaged by intraperitoneal injection of spleen homogenates in Hartley guinea pigs seven times. Immunized guinea pigs were injected intraperitoneally with 0.5 ml of a 1:1,000 dilution of spleen cell homogenate in Hank's balanced salt solution 122 days after the initial plasmid DNA injection (1000 pfu). Survival was determined 10 days later at which times animals were sacrificed for serologic and pathologic analysis. ELISA, enzyme-linked immunosorbent assay (Volchkov, V.E. et al., *FEBS. Lett.* 305:181-184 (1992); Sanchez, A. et al., *Virus. Res.* 29:215-240 (1993)) on infected

cell supernatants and enriched viral extracts containing GP, sGP, or NP were performed as previously described.

III. DISCUSSION

Following the initial report that injection of plasmid DNA into muscle could direct the synthesis of recombinant proteins (Wolff, J.A. et al., *Science* 247:1465-1468 (1990)), the suggestion was made that this gene transfer approach may be useful for vaccination and was termed genetic immunization. Tang, D.C. et al., *Nature* 356:152-154 (1992). This approach has been applied to different infectious diseases, including influenza (Ulmer, J.B. et al., *Science* 259:1745-1749 (1993); Raz, E. et al., *PNAS (USA)* 91:9519-9523 (1994)), malaria (Doolan, D.L. et al., *J. Exp. Med.* 183:1739-1746 (1996); Sedegah, M. et al., *PNAS (USA)* 91:9866-9870 (1994)), and tuberculosis (Tascon, R.C. et al., *Nat. Med.* 2:888-892 (1996)) and has also been used to modulate antibody and cell-mediated immune responses in autoimmune and allergic diseases. Raz, E. et al., *PNAS (USA)* 90:4523-4527 (1993); Waisman, A. et al., *Nat. Med.* 2:899-905 (1996); McCormick, J.B. et al., *J. Infect. Dis.* 147:264-267 (1983); Border, W.A. et al., *Nat. Med.* 1:1000-1001 (1995).

The immune response to selected Ebola virus proteins after genetic immunization in mice was analyzed and their ability to protect against lethal infection in a susceptible animal model, the guinea pig, was tested. The immune analyses performed in different species suggest similar patterns of response, though the specific peptides which may be recognized by the immune system to confer protection in the guinea pig could differ from the mouse. Because the principles of MHC antigen presentation and recognition apply broadly across species (Monaco, J.J., *Immunol. Today* 13:173-179 (1992); Jorgensen, J.L. et al., *Annu. Rev. Immunol.* 10:835-873 (1992); Zinkernagel, R.M. et al., *Immunol. Today* 18:14-17 (1997)), the finding that protection was observed in different members of an outbred strain and that similar immune responses were seen in different species is not unexpected and suggests that this approach may be applicable to humans.

Immunization with plasmids encoding distinct viral proteins induced different antibody and cytolytic T cell responses. The broadest immune response was conferred by GP and sGP, which induced both cellular and humoral immunity to the membrane-associated GP. In guinea pigs challenged with doses of virus that are otherwise lethal, sGP provided nearly equivalent protection to GP, with no significant difference between these groups. The ability of vectors expressing GP to confer immunity may be explained by the generation of lower molecular weight degradation

products (Fig. 1B) which could provide sufficient protein for antigen presentation to induce detectable, cellular, and humoral immune responses in guinea pigs.

Despite the fact that plasmid DNA injection has been shown to affect the immune response to different antigens in infectious and autoimmune diseases, the ability of individual gene products to protect against disease *in vivo* is not readily predictable. In particular, the rapid rates of Ebola virus replication and the poor immunogenicity of its proteins had previously rendered it resistant to immune interventions. Several attempts to confer protection with passive transfer of immunoglobulin were unsuccessful (Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Jahrling, P.B. et al., *Arch. Virol. Suppl.* 11:135-140 (1996)), in agreement with the finding set forth herein that antisera from protected animals fails to neutralize virus replication *in vitro*. Previous studies using formalin-fixed virus or purified viral proteins for immunization have also not proven effective. Peters, C.J. et al., *Filoviridae*: Marburg and Ebola Viruses. in *Fields Virology*. (eds., Fields, B.N., Knipe, D.M.& Howley, P.M.) 1161-1176 (Philadelphia, Lippincott-Raven, 1996); Clegg, J.C.S. & Sanchez, A. Vaccines against arenaviruses and filoviruses. in *New Generation Vaccines*. (eds., Levine, M.M., Woodrow, G.C., Kaper, J.B.& Cobon, G.S.) 749-765 (New York, NY, Marcel Dekker, Inc. 1997).

It is likely that traditional immunization approaches using protein antigens, vaccinia virus, or inactivated virus do not allow for appropriate uptake and presentation of viral antigens by dendritic or other antigen-presenting cells to induce protective immune responses. It has been shown recently that genetic immunization leads to production of recombinant protein(s) in muscle which are delivered to bone marrow-derived antigen-presenting cells. Iwasaki, A. et al., *J. Immunol.* 159:11-14 (1997); Doe, B. et al., *PNAS (USA)* 93:8578-8583 (1996); Corr, M. et al., *J. Exp. Med.* 184:1555-1560 (1996). Synthesis of Ebola glycoprotein after gene transfer apparently allows more efficient processing and presentation and the generation of immune responses not seen with virus or with viral vectors. GP is a large molecule which contains both T and B cell epitopes. Although antibody levels provide a surrogate marker of protection, the fact that passive transfer of antibody did not confer protection implies that immunoglobulin switching and synthesis is reflective of the T helper response to GP. Genetic immunization stimulates T helper cells to generate both CTL and B cell antibody responses to the virus. Although antibody production confirms effective immunization, a productive T cell response, likely involving T_H1 cell

stimulation, as shown by the T cell proliferation and CTL assays (Fig. 3), is needed for effective immunity. Taken together, these studies suggest that transcription and translation of viral genes in host cells by genetic immunization induces alternative, more effective, processing and antigen presentation which better stimulates immunity to Ebola virus. Since there are yet no effective antiviral agents, the ability to generate protective immunity by vaccination may prove useful in selected high risk populations, particularly in regions of ongoing outbreaks, and among medical and laboratory personnel exposed to the virus. Although it remains important to identify agents which treat acute infection, genetic immunization may help to limit the spread of this highly lethal infectious disease.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

All references cited herein are incorporated by reference as if fully set forth.

WE CLAIM:

1. A pharmaceutical composition comprising a nucleic acid molecule encoding an Ebola virus structural gene product operatively-liked to a control sequence, in a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of Claim 1, wherein the Ebola virus structural gene product is selected from the group consisting of the transmembrane form of virus glycoprotein, the secreted form of virus glycoprotein, virus nucleoprotein and combinations thereof.

3. The pharmaceutical composition of Claim 1, wherein the control sequence is a promoter.

4. The pharmaceutical composition of Claim 3, wherein the promoter is the CMV immediate-early region 1 promoter.

5. The pharmaceutical composition of Claim 1, further comprising an adjuvant.

6. The pharmaceutical composition of Claim 2, wherein the structural gene product is the transmembrane form of virus glycoprotein.

7. The pharmaceutical composition of Claim 2, wherein the structural gene product is the secreted form of virus glycoprotein.

8. The pharmaceutical composition of Claim 2, wherein the structural gene product is virus nucleoprotein.

9. A method of producing a vaccine against disease caused by infection by Ebola virus, comprising the steps of:

a) administering the pharmaceutical composition of Claim 1 to a test host to determine an amount and a frequency of administration thereof to elicit a protective immune response in said host; and

b) formulating said pharmaceutical composition in a form suitable for administration to a treatable host in accordance with said determined amount and frequency of administration.

10. A vaccine comprising a nucleic acid molecule encoding the transmembrane form of the Ebola virus glycoprotein operatively-linked to a control sequence, in a pharmaceutically acceptable carrier.

11. The vaccine of Claim 10, wherein the control sequence is a promoter.

12. The vaccine of Claim 11, wherein the promoter is the CMV immediate-early region 1 promoter.

13. The vaccine of Claim 10, further comprising an adjuvant.

14. A vaccine comprising a nucleic acid molecule encoding the secreted form of the Ebola virus glycoprotein operatively-linked to a control sequence, in a pharmaceutically acceptable carrier.

15. The vaccine of Claim 14, wherein the control sequence is a promoter.

16. The vaccine of Claim 15, wherein the promoter is the CMV immediate-early region 1 promoter.

17. The vaccine of Claim 14, further comprising an adjuvant.

18. A vaccine comprising a nucleic acid molecule encoding the Ebola virus nucleoprotein operatively-linked to a control sequence, in a pharmaceutically acceptable carrier.

19. The vaccine of Claim 18, wherein the control sequence is a promoter.

20. The vaccine of Claim 19, wherein the promoter is the CMV immediate-early region 1 promoter.

21. The vaccine of Claim 18, further comprising an adjuvant.

22. A method of immunizing a subject against hemorrhagic fever comprising the step of administering to the host an immunoeffective amount of the vaccine of any of Claims 10 to 21.

23. The method of Claim 22, wherein the hemorrhagic fever is caused by infection with Ebola virus.

24. The method of Claim 22, wherein the hemorrhagic fever is caused by infection with Marburg virus.

25. The method of Claim 22, wherein the host is a human and administration is by intramuscular injection.

26. The method of Claim 22, wherein the subject receives a second administration of an immunoeffective amount of a vaccine against disease caused by infection by Ebola virus or Marburg virus.

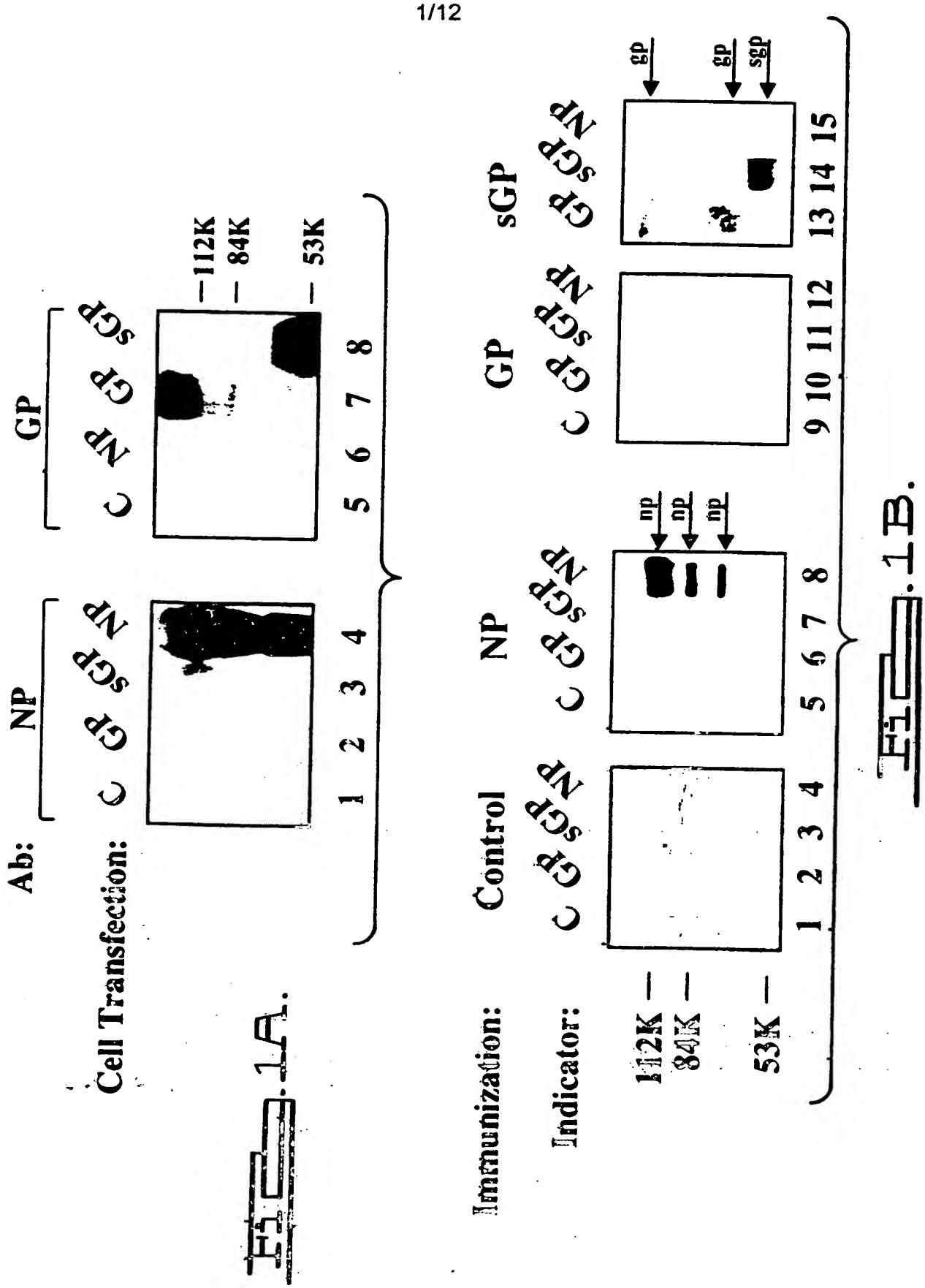

FIG. 1A.

FIG. 1B.

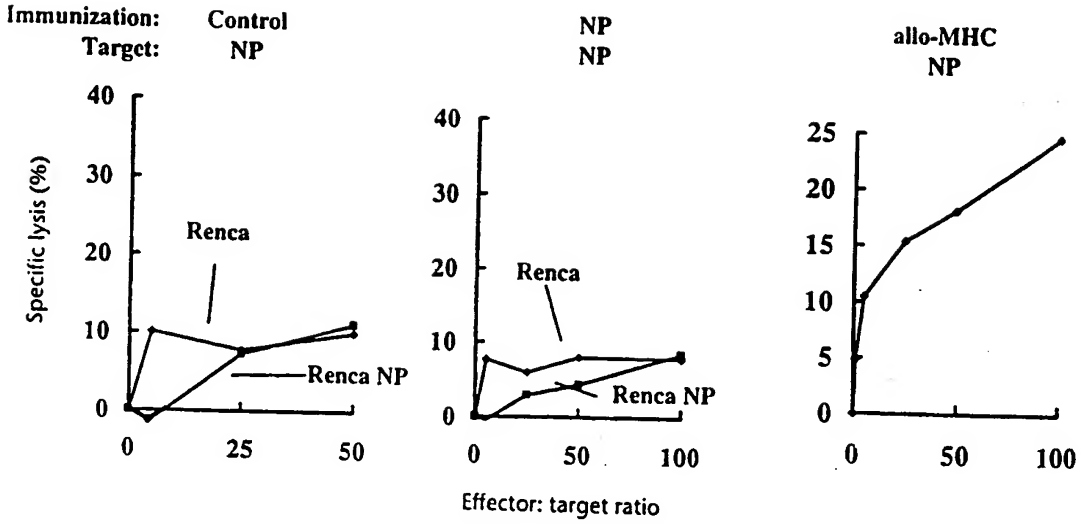

FIGURE 2A

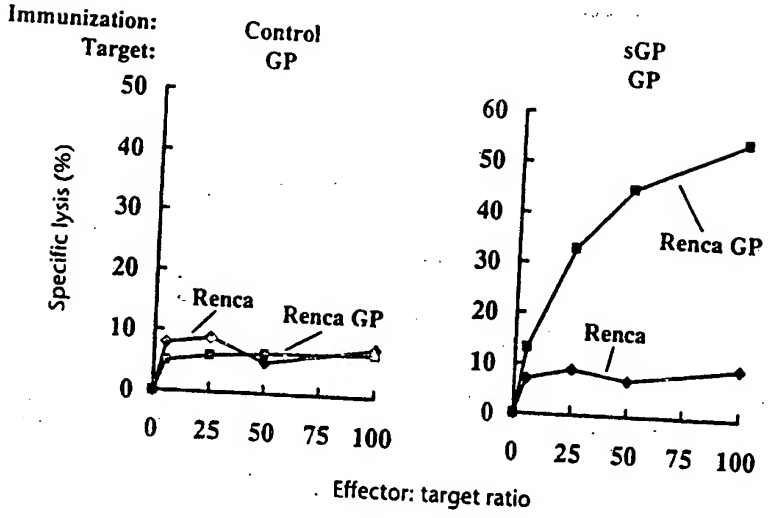

FIGURE 2B

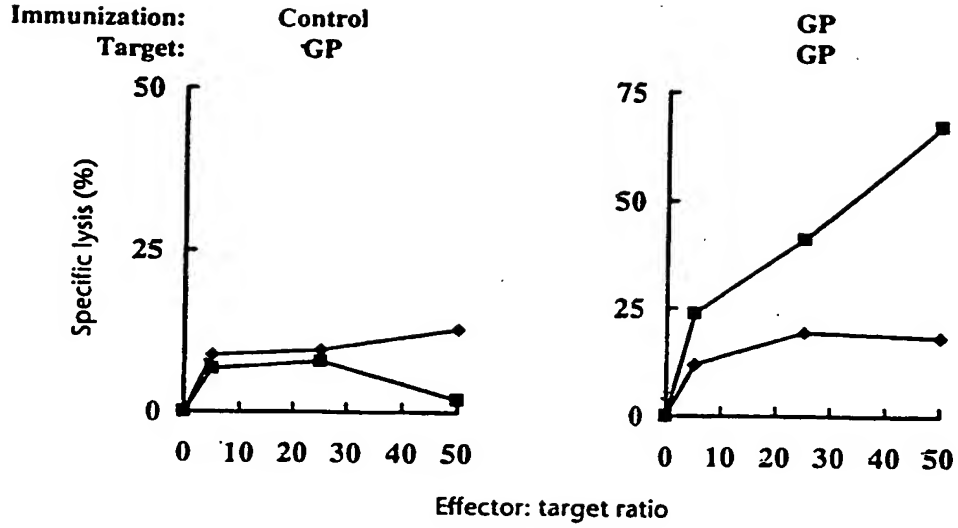

FIGURE 2C

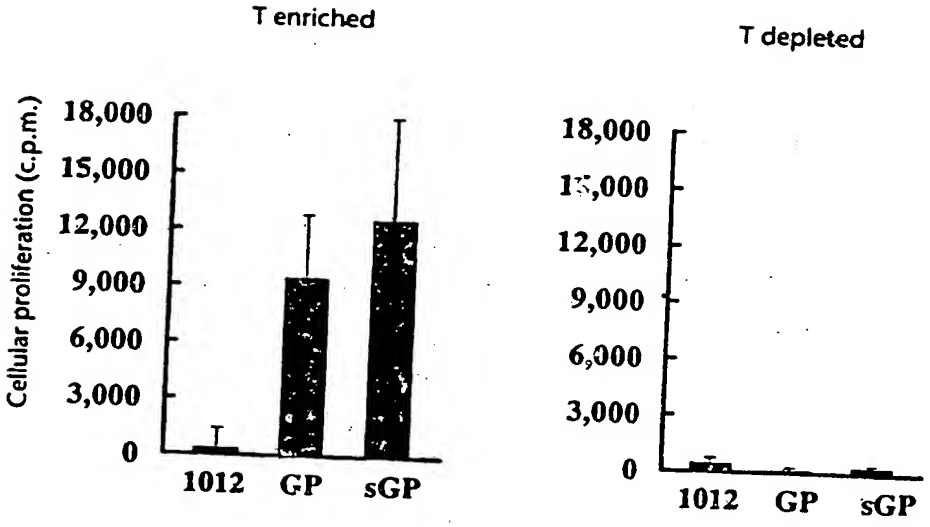

FIGURE 2D

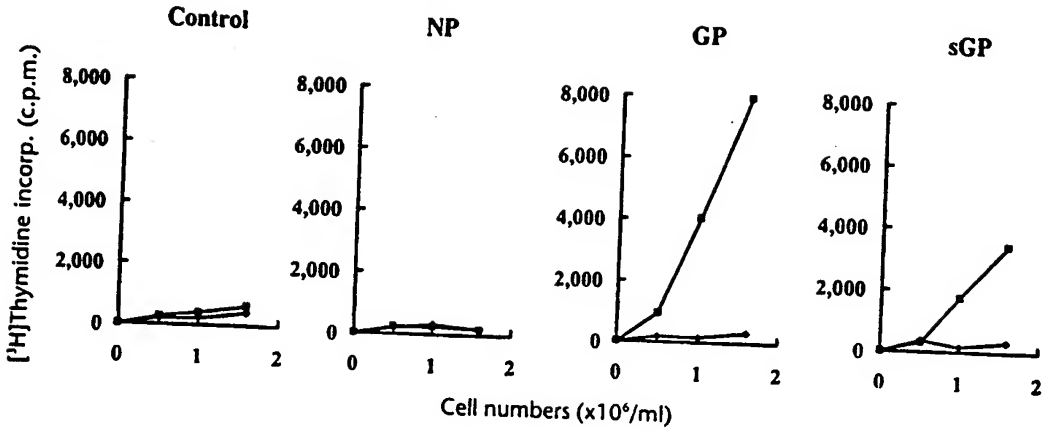

FIGURE 3A

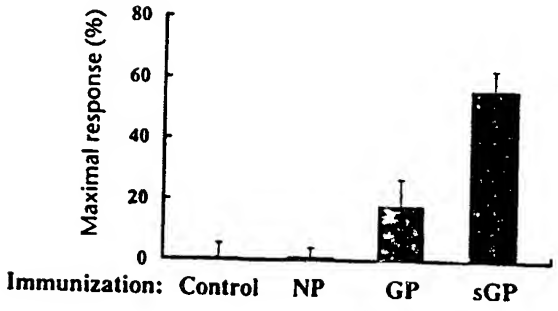

FIGURE 3B

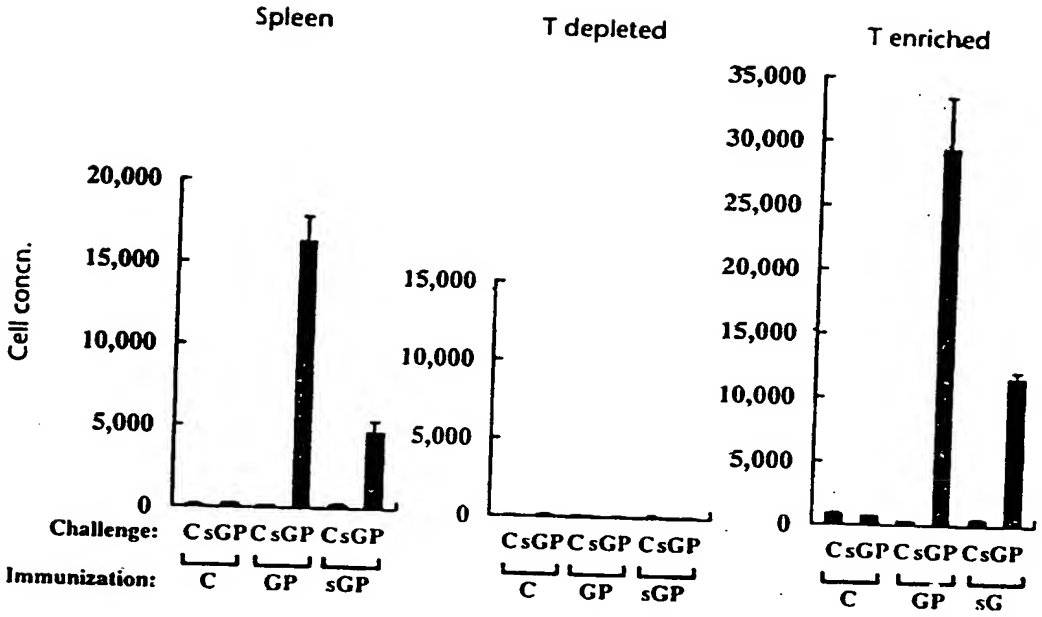

FIGURE 3C

Protected

Liver:

Fig. 4A.

Lung:

Fig. 4C.

Spleen:

Fig. 4E.

Infected

Liver:

Fig. 4B.

Lung:

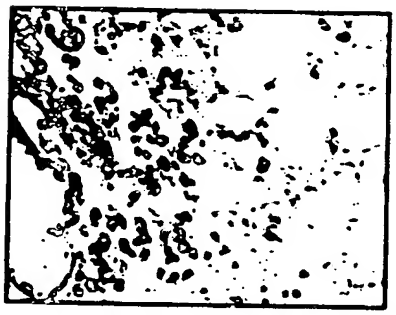

Fig. 4D.

Spleen:

Fig. 4F.

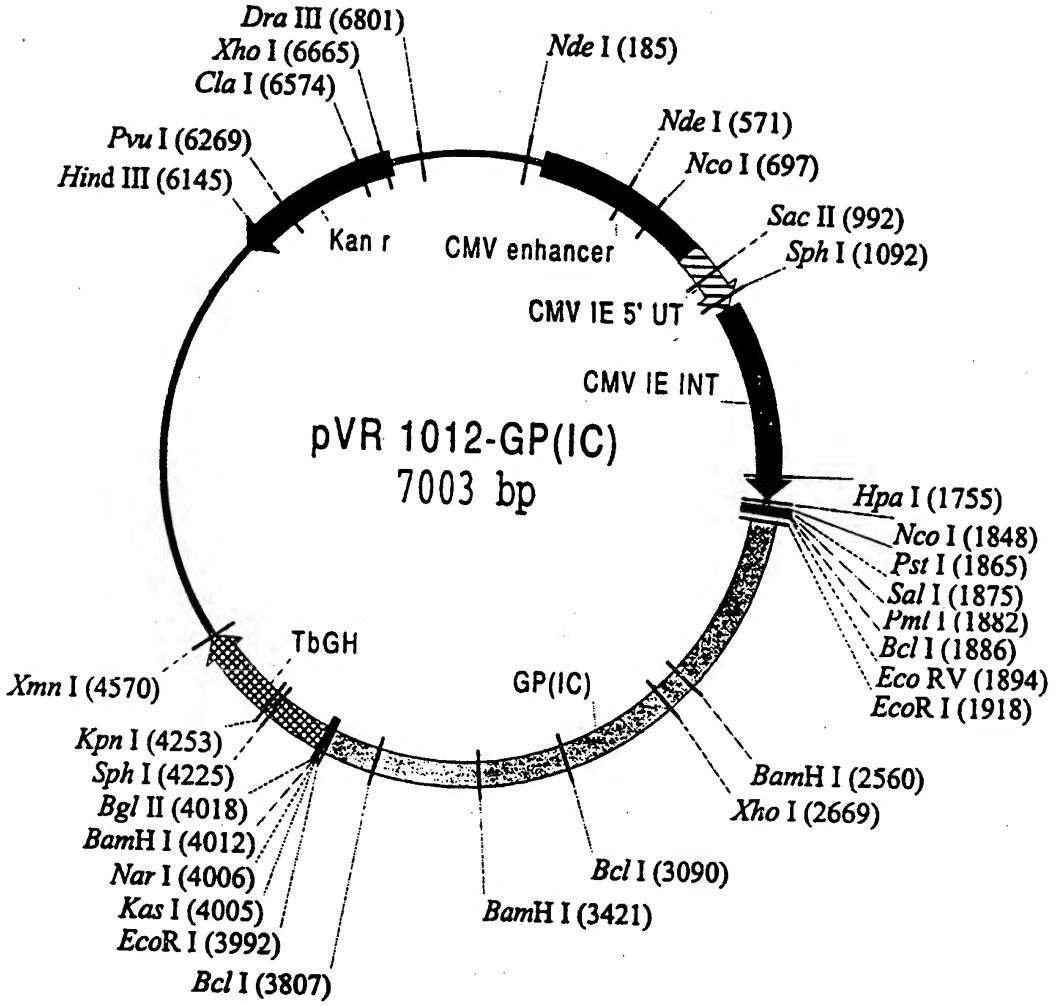

FIGURE 5

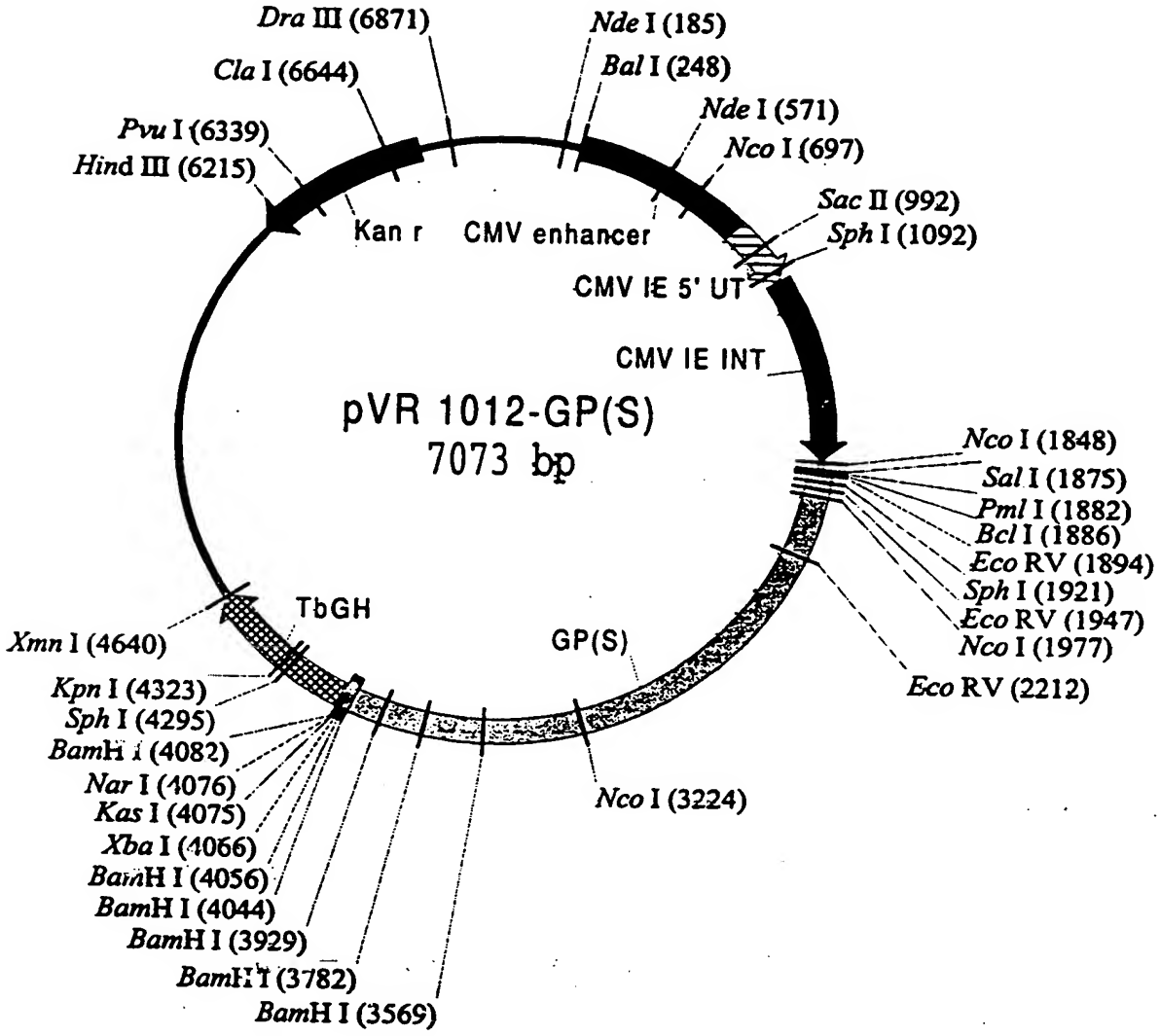

FIGURE 6

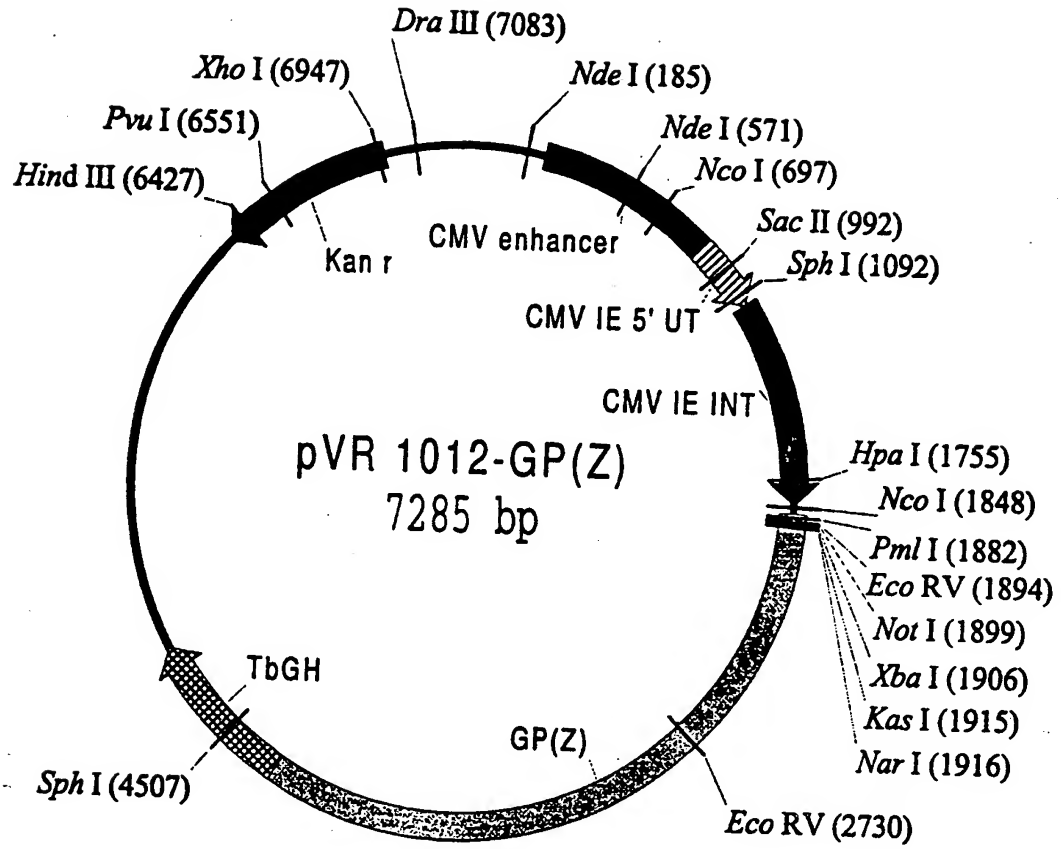

FIGURE 7

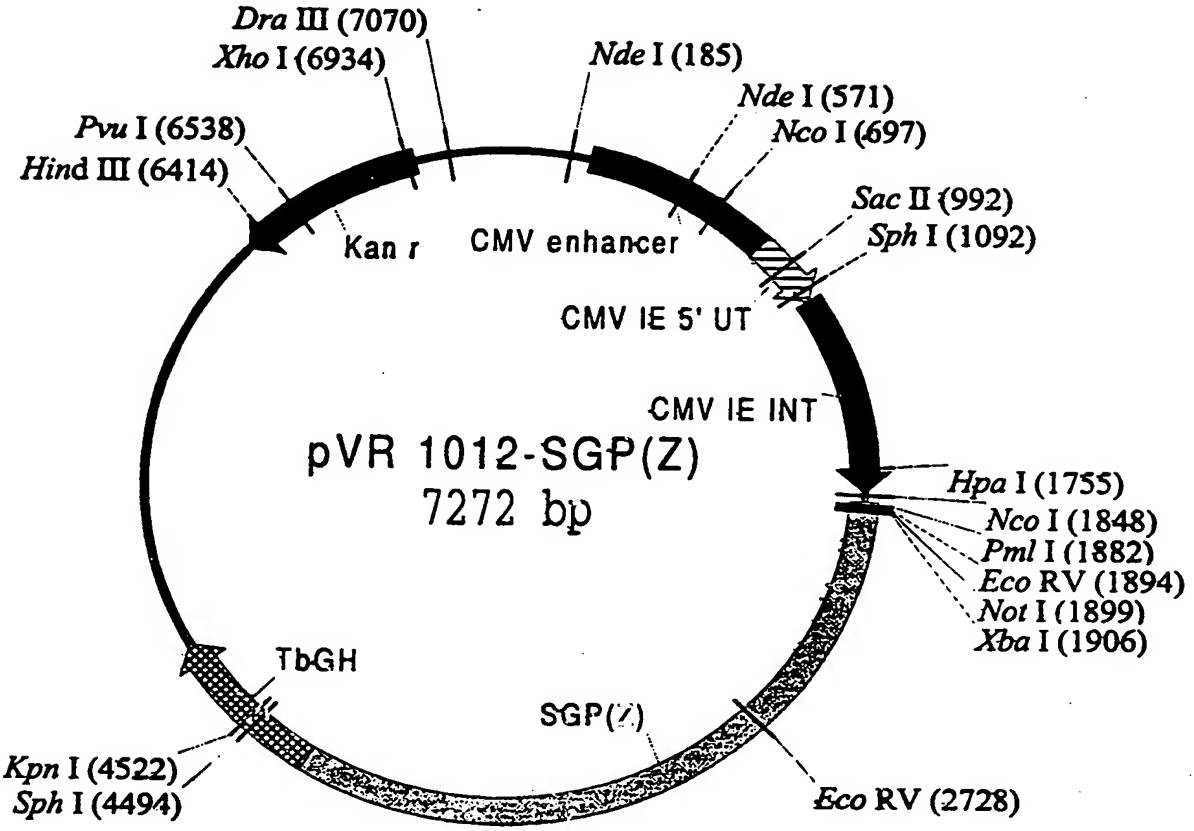

FIGURE 8

Number: 699 Name: VR1012-NP Lab member: Ling
Backbone origin: [unknown] Constr. date: [unknown] Length(bp): [unknown]
Keywords: [non]
Comments: [none]

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No MacPlasmap file available online
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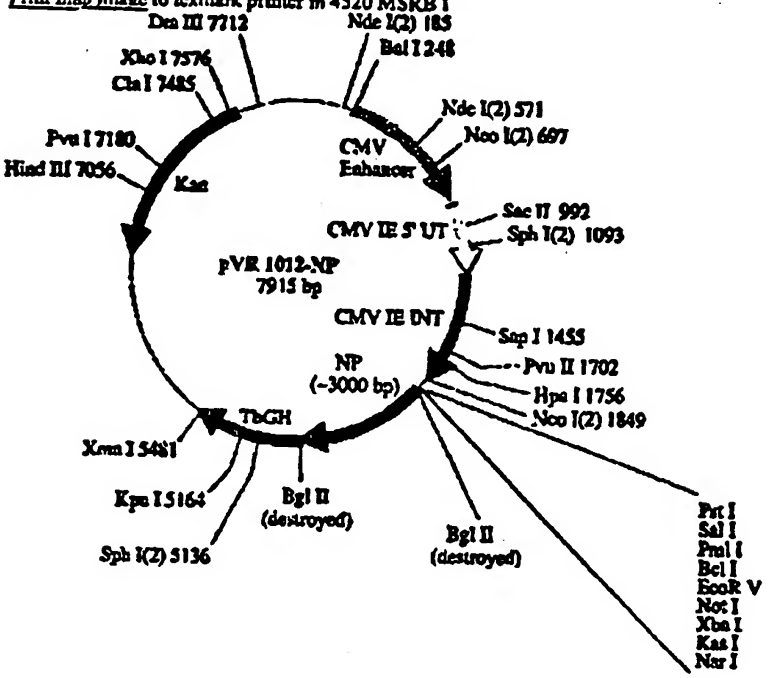

Plasmid name: pVR 1012-NP
Plasmid size: 7915 bp
Constructed by: Ling
Construction date: 1994
Comments/References: none

Figure 9

pVR 1012-GP(IC)

Sequence Listing ID No: 1

General Description

DNA pVR 1012-GP(IC)
Local object
Created: 09/14/98 04:17PM
Last Modification Date: ? (no data)
length: 7003 bp
storage type: Basic
form: Circular

Comments

Restriction Map

BglII: 1 site AGATCT / TCTAGA

ClaI: 1 site ATCGAT / TAGCTA

DraIII: 1 site CACNNNGTG / GTGNNNCAC

EcoRV: 1 site GATATC / CTATAG

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KasI: 1 site GGCGCC / CCGCGG

KpnI: 1 site GGTACC / CCATGG

NarI: 1 site GGCGCC / CCGCGG

PmlI: 1 site CACGTG / GTGCAC

PstI: 1 site CTGCAG / GACGTC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

SalI: 1 site GTCGAC / CAGCTG

XmnI: 1 site GAANNNNTTC / CTTNNNNAAG

EcoRI: 2 sites GAATTC / CTTAAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

XhoI: 2 sites CTCGAG / GAGCTC

BamHI: 3 sites GGATCC / CCTAGG

BclI: 3 sites TGATCA / ACTAGT

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1840

TbGH
Start: 4020 End: 4572

Kan r
Start: 6068 End: 6690 (Complementary)

Misc_feature (2 signals)

CMV enhancer
Start: 248 End: 885

GP(IC)
Start: 1870 End: 4019

Annotations

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   1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
      AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

  51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
      CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCGCTCGT CTGTTCGGGC

 101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
      AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                          NdeI
 151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
      GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

 201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
      GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

 251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
      AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

 301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
      AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

 351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
      TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

 401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
      TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

 451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
      GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

 501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
      GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                      NdeI
 551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
      CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

 601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
      ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                        NcoI
 651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
      TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

      NcoI
      -
 701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
      CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

 751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
      TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

 801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
      CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 ....................................................................................

 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
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 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
 ....................................................................................

1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
 ....................................................................................

                                                 SphI
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1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
 ....................................................................................

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
 ....................................................................................

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
 ....................................................................................

1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
 ....................................................................................

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
 ....................................................................................

1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
 ....................................................................................

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
 ....................................................................................

1401  GCGTCGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCAGCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
 ....................................................................................

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
 ....................................................................................

1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
 ....................................................................................

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGCCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
 ....................................................................................

1601  TCGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
 ....................................................................................

1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
 ....................................................................................

1701  CCGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
 ....................................................................................
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HpaI

1751 TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCGGCG
ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

NcoI

1801 CGCGCCAGCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

SalI

NcoI PstI PmlI BclI EcoRV

1851 GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

EcoRI

1901 CCGCGCGGCC GCTCTAGAAT TCTCTAATCA CAGTCATCAT GGGAGCGTCA
GGCGCGCCGG CGAGATCTTA AGAGATTAGT GTCAGTAGTA CCCTCGCAGT

1951 GGGATTCTGC AATTGCCGCG TGAGCGCTTC ACGAAAACAT CTTTCTTTGT
CCCTAAGACG TTAACGGGGC ACTCGCGAAG TGCTTTTGTA GAAAGAAACA

2001 TTGGGTAATA ATCCTATTCC ATAAAGTCTT TTCAATCCCG TTGGGGGTTG
AACCCATTAT TAGGATAAGG TATTTCAGAA AAGTTAGGGC AACCCCCAAC

2051 TACACAACAA TACCCTACAA GTGAGTGATA TTGACAAGTT TGTGTGCCGA
ATGTGTTGTT ATGGGATGTT CACTCACTAT AACTGTTCAA ACACACGGCT

2101 GACAAACTCT CTTCAACTAG CCAATTGAAC TCAGTCGGGT TGAACTTGGA
CTGTTTGAGA GAAGTTGATC GGTTAACTTC AGTCAGCCCA ACTTGAACCT

2151 GGGCAATGGA GTAGCAACTG ATGTACCAAC GGCAACCAAA AGATCGGGTT
CCCGTTACCT CATCGTTGAC TACATGGTTG CCGTTGGTTT TCTAGCCCAA

2201 TTCGAGCTGG TGTTCCACCA AAGGTGGTAA ATTACGAAGC TGGAGAATGG
AAGCTCGACC ACAAGGTGGT TTCCACCATT TAATGCTTCG ACCTCTTACC

2251 GCTGAGAACT GTTATAACCT GGCTATAAAG AAAGTTGATG GTAGTGAGTG
CGACTCTTGA CAATATTGGA CCGATATTTC TTTCAACTAC CATCACTCAC

2301 CCTACCAGAA GCCCCTGAGG GAGTGAGGGA TTTTCGCCGT TGCCGCTATG
GGATGGTCTT CGGGGACTCC CTCACTCCCT AAAAGGGGCA ACGGCGATAC

2351 TACACAAAGT CTCAGGAACT GGACCATGCC CAGGAGGACT CGCCTTTCAC
ATGTGTTTCA GAGTCCTTGA CCTGGTACGG GTCCTCCTGA GCGGAAAGTG

2401 AAAGAAGGAG CCTTCTTCCT GTATGACCGA CTCGCATCAA CAATCATTTA
TTTCTTCCTC GGAAGAAGGA CATACTGGCT GAGCGTAGTT GTTAGTAAAT

2451 TCGGGGTACA ACCTTTGCCG AAGGAGTTAT TGCATTTCTG ATCTTGCCTA
AGCCCCATGT TGGAAACGGC TTCCTCAATA ACGTAAAGAC TAGAACGGAT

2501 AGGCGCGAAA GGATTTTTTC CAGTCTGCTC CATTGCATGA GGCTGCCAAC
TCCGCGCTTT CCTAAAAAAG GTCAGACGAG GTAACGTACT CCGACGGTTG

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                BamHI
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2551  ATGACCACGG ATCCCTCCAG TTACTATCAC ACGACAACAA TAAACTACGT
      TACTGGTGCC TAGGGAGGTC AATGATAGTG TGCTGTTGTT ATTTGATGCA
 .....
2601  GGTTGATAAT TTTGGAACCA ACACCACAGA GTTTCTGTTC CAAGTCGATC
      CCAACTATTA AAACCTTGGT TGTGGTGTCT CAAAGACAAG GTTCAGCTAG
 .....
                        XhoI
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2651  ATTTGACGTA TGTGCAGCTC GAGGCAAGAT TCACACCACA ATTCCTTGTC
      TAAACTGCAT ACACGTCGAG CTCCGTTCTA AGTGTGGTGT TAAGGAACAG
 .....
2701  CTCCTAAATG AAACCATCTA CTCTGATAAC CGCAGAAGTA ACACAACAGG
      GAGGATTTAC TTTGGTAGAT GAGACTATTG GCGTCTTCAT TGTGTTGTCC
 .....
2751  AAAACTAATC TGGAAAATAA ATCCCACTGT TGATACCAGC ATGGGTGAGT
      TTTTGATTAG ACCTTTTATT TAGGGTGACA ACTATGGTCG TACCCACTCA
 .....
2801  GGGCTTTCTG GGAAAATAAA AAAACTTCAC AAAAACCCTT TCAAGTGAAG
      CCCGAAAGAC CCTTTTATTT TTTTGAAGTG TTTTTGGGAA AGTTCACTTC
 .....
2851  AGTTGTCTTT CGTACCTGTA CCAGAAACCC AGAACCAGGT CCTTGACACG
      TCAACAGAAA GCATGGACAT GGTCTTTGGG TCTTGGTCCA GGAACTGTGC
 .....
2901  ACAGCGACGG TCTCTCCTCC CATCTCCGCC CACAACCACG CAGGCGAAGA
      TGTCGCTGCC AGAGAGGAGG GTAGAGGCGG GTGTTGGTGC GTCCGCTTCT
 .....
2951  CCACAAAGAA TTGGTTTCAG AGGATTCCAC TCCAGTGGTT CAGATGCAAA
      GGTGTTTCTT AACCAAAGTC TCCTAAGGTG AGGTCACCAA GTCTACGTTT
 .....
3001  ACATCAAGGG AAAGGACACA ATGCCAACCA CAGTGACGGG TGTACCAACA
      TGTACTTCCC TTTCCTGTGT TACGGTTGGT GTCACTGCCC ACATGGTTGT
 .....
                                              BclI
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3051  ACCACACCCT CTCCATTTCC AATCAATGCT CGCAACACTG ATCATACCAA
      TGGTGTGGGA GAGGTAAAGG TTAGTTACGA GCGTTGTGAC TAGTATGGTT
 .....
3101  ATCATTTATC GGCCTGGAGG GGCCCCAAGA AGACCACAGC ACCACACAGC
      TAGTAAATAG CCGGACCTCC CCGGGGTTCT TCTGGTGTCG TGGTGTGTCG
 .....
3151  CTGCCAAGAC CACCAGCCAA CCAACCAACA GCACAGAATC GACGACACTA
      GACGGTTCTG GTGGTCGGTT GGTTGGTTGT CGTGTCTTAG CTGCTGTGAT
 .....
3201  AACCCAACAT CAGAGCCCTC CAGTAGAGGC ACGGGACCAT CCAGCCCCAC
      TTGGGTTGTA GTCTCGGGAG GTCATCTCCG TGCCCTGGTA GGTCGGGGTG
 .....
3251  GGTCCCGAAC ACCACAGAAA GCCACGCCGA ACTTGGCAAG ACAACCCCAA
      CCAGGGGTTG TGGTGTCTTT CGGTGCGGCT TGAACCGTTC TGTTGGGGTT
 .....
3301  CCACACTCCC AGAACAGCAC ACTGCCGCCA GTGCCATTCC AAGAGCCGTG
      GGTGTGAGGG TCTTGTCGTG TGACGGCGGT CACGGTAAGG TTCTCGGCAC
 .....
3351  CACCCCGACG AACTCAGTGG ACCTGGCTTC CTGACGAACA CAATACGGGG
      GTGGGGCTGC TTGAGTCACC TGGACCGAAG GACTGCTTGT GTTATGCCCC
 .....
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                          BamHI
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3401  GGTGACAAAT CTCCTGACAG GATCCAGAAG AAAGCGAAGG GATGTCACTC
      CCACTGTTTA GAGGACTGTC CTAGGTCTTC TTTCGCTTCC CTACAGTGAG
      ..........................................................
3451  CCAATACACA ACCCAAATGC AACCCAAACC TGCACTATTG GACAGCCTTG
      GGTTATGTGT TGGGTTTACG TTGGGTTTGG ACGTGATAAC CTGTCGGAAC
      ..........................................................
3501  GATGAGGGTG CTGCCATAGG TTTAGCCTGG ATACCATACT TCGGGCCAGC
      CTACTCCCAC GACGGTATCC AAATCGGACC TATGGTATGA AGCCCGGTCG
      ..........................................................
3551  AGCTGAGGGA ATTTACACTG AAGGCATAAT CGAGAATCAA AATGGATTGA
      TCGACTCCCT TAAATGTGAC TTCCGTATTA GCTCTTAGTT TTACCTAACT
      ..........................................................
3601  TCTGTGGATT GAGGCAGCTG GCCAACGAAA CGACACAAGC TCTTCAATTG
      AGACACCTAA CTCCGTCGAC CGGTTGCTTT GCTGTGTTCG AGAAGTTAAC
      ..........................................................
3651  TTCTTAAGGG CAACTACTGA GTTGCGTACA TTCTCTATAC TAAATCGGAA
      AAGAATTCCC GTTGATGACT CAACGCATGT AAGAGATATG ATTTAGCCTT
      ..........................................................
3701  AGCAATAGAC TTCTTGCTCC AAAGATGGGG AGGAACATGT CACATTCTAG
      TCGTTATCTG AAGAACGAGG TTTCTACCCC TCCTTGTACA GTGTAAGATC
      ..........................................................
3751  GGCCTGATTG TTGCATTGAA CCCCAAGATT GGAGCAAAAA TATCACTGAT
      CCGGACTAAC AACGTAACTT GGGGTTCTAA CCTCGTTTTT ATAGTGACTA
      ..........................................................
            BclI
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3801  AAAATTGATC AAATAATCCA TGACTTTGTC GATAATAATC TTCCAAATCA
      TTTTAACTAG TTTATTAGGT ACTGAAACAG CTATTATTAG AAGGTTTAGT
      ..........................................................
3851  GAATGATGGC AGCAACTGGT GGACTGGATG GAAACAATGG GTTCCTGCTG
      CTTACTACCG TCGTTGACCA CCTGACCTAC CTTTGTTACC CAAGGACGAC
      ..........................................................
3901  GAATAGGAAT CACACGAGTA ATCATTGCTA TTATTGCTTT GCTGTACATT
      CTTATCCTTA GTGTGCTCAT TAGTAACGAT AATAACGAAA CGACACGTAA
      ..........................................................
                                                    EcoRI
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3951  TGCAAATTCA TGCTTTGAAC TAATATAGCA TCATACTTTA GAATTCTAGA
      ACGTTTAAGT ACGAAACTTG ATTATATCGT AGTATGAAAT CTTAAGATCT
      ..........................................................
         NarI
         ----
         XbaI     BlnHI BglII
         ----     ------ -----
4001  CCAGGCGCCT GGATCCAGAT CTGCTGTGCC TTCTAGTTGC CAGCCATCTG
      GGTCCGCGGA CCTAGGTCTA GACGACACGG AAGATCAACG GTCGGTAGAC
      ..........................................................
4051  TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC
      AACAAACGGG GAGGGGGCAC GGAAGGAACT GGGACCTTCC ACGGTGAGGG
      ..........................................................
4101  ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG
      TGACAGGAAA GGATTATTTT ACTCCTTTAA CGTAGCGTAA CAGACTCATC
      ..........................................................
4151  GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGCACAGC AAGGGGGAGG
      CACAGTAAGA TAATGGCCCC CACCCCACCC CGTCGTGTCC TTCCCCCTCC
      ..........................................................
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SphI KpnI

4201 ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGGT
TAACCCTTCT GTTATCGTCC GTACGACCCC TACGCCACCC GAGATACCCA

KpnI

4251 ACCCAGGTGC TGAAGAATTG ACCCGGTTCC TCCTGGGCCA GAAAGAAGCA
TGGGTCCACG ACTTCTTAAC TGGGCCAAGG AGGACCCGGT CTTTCTTCGT

4301 GGCACATCCC CTTCTCTGTG ACACACCCTG TCCACGCCCC TGGTTCTTAG
CCGTGTAGGG GAAGAGACAC TGTGTGGGAC AGGTGCGGGG ACCAAGAATC

4351 TTCCAGCCCC ACTCATAGGA CACTCATAGC TCAGGAGGGC TCCGCCTTCA
AAGGTCGGGG TGAGTATCCT GTGAGTATCG AGTCCTCCCG AGGCGGAAGT

4401 ATCCCACCCG CTAAAGTACT TGGAGCGGTC TCTCCCTCCC TCATCAGCCC
TAGGGTGGGC GATTTCATGA ACCTCGCCAG AGAGGGAGGG AGTAGTCGGG

4451 ACCAAACCAA ACCTAGCCTC CAAGAGTGGG AAGAAATTAA AGCAAGATAG
TGGTTTGGTT TGGATCGGAG GTTCTCACCC TTCTTTAATT TCGTTCTATC

4501 GCTATTAAGT GCAGAGGGAG AGAAAATGCC TCCAACATGT GAGGAAGTAA
CGATAATTCA CGTCTCCCTC TCTTTTACGG AGGTTGTACA CTCCTTCATT

XmnI

4551 TGAGAGAAAT CATAGAATTT CTTCCGCTTC CTCGCTCACT GACTCGCTGC
ACTCTCTTTA GTATCTTAAA GAAGGCGAAG GAGCGAGTGA CTGAGCGACG

4601 GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA
CGAGCCAGCA AGCCGACGCC GCTCGCCATA GTCGAGTGAG TTTCCGCCAT

4651 ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC
TATGCCAATA GGTGTCTTAG TCCCCTATTG CGTCCTTTCT TGTACACTCG

4701 AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
TTTTCCGGTC GTTTTCCGGT CCTTGGCATT TTTCCGGCGC AACGACCGCA

4751 TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA
AAAAGGTATC CGAGGCGGGG GGACTGCTCG TAGTGTTTTT AGCTGCGAGT

4801 AGTCAGAGGT GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC
TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT ATTTCTATGG TCCGCAAAGG

4851 CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG
GGGACCTTCG AGGGAGCACG CGAGAGGACA AGGCTGGGAC GGCGAATGGC

4901 GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC
CTATGGACAG GCGGAAAGAG GGAAGCCCTT CGCACCGCGA AAGAGTTACG

4951 TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG
AGTGCGACAT CCATAGAGTC AAGCCACATC CAGCAAGCGA GGTTCGACCC

5001 CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
GACACACGTG CTTGGGGGGC AAGTCGGGCT GGCGACGCGG AATAGGCCAT

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5051  ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA
      TGATAGCAGA ACTCAGGTTG GGCCATTCTG TGCTGAATAG CGGTGACCGT
.....
5101  GCAGCCACTG GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC
      CGTCGGTGAC CATTGTCCTA ATCGTCTCGC TCCATACATC CGCCACGATG
.....
5151  AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT
      TCTCAAGAAC TTCACCACCG GATTGATGCC GATGTGATCT TCCTGTCATA
.....
5201  TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT
      AACCATAGAC GCGAGACGAC TTCGGTCAAT GGAAGCCTTT TTCTCAACCA
.....
5251  AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT
      TCGAGAACTA GGCCGTTTGT TTGGTGGCGA CCATCGCCAC CAAAAAAACA
.....
5301  TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
      AACGTTCGTC GTCTAATGCG CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA
.....
5351  TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA
      ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA CCTTGCTTTT GAGTGCAATT
.....
5401  GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT
      CCCTAAAACC AGTACTCTAA TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA
.....
5451  AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT
      TTTAATTTTT ACTTCAAAAT TTAGTTAGAT TTCATATATA CTCATTTGAA
.....
5501  CGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC
      CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA GAGTCGCTAG
.....
5551  TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCGGGGGG GGGGGGCGCT
      ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGCCCCCC CCCCCCGCGA
.....
5601  GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG ACTCATACCA GGCCTGAATC
      CTCCAGACGG AGCACTTCTT CCACAACGAC TGAGTATGGT CCGGACTTAG
.....
5651  GCCCCATCAT CCAGCCAGAA AGTGAGGGAG CCACGGTTGA TGAGAGCTTT
      CGGGGTAGTA GGTCGGTCTT TCACTCCCTC GGTGCCAACT ACTCTCGAAA
.....
5701  GTTGTAGGTG GACCAGTTGG TGATTTTGAA CTTTTGCTTT GCCACGGAAC
      CAACATCCAC CTGGTCAACC ACTAAAACTT GAAAACGAAA CGGTGCCTTG
.....
5751  GGTCTGCGTT GTCGGGAAGA TGCGTGATGT GATGCTTCAA CTCAGCAAAA
      CCAGACGCAA CAGCCCTTCT ACGCACTAGA CTACGAAGTT GAGTCGTTTT
.....
5801  GTTCGATTTA TTCAACAAAG CCGCCGTCCC GTCAAGTCAG CGTAATGCTC
      CAAGCTAAAT AAGTTGTTTC GGCGGCAGGG CAGTTCAGTC GCATTACGAG
.....
5851  TGCCAGTGTT ACAACCAATT AACCAATTCT GATTAGAAAA ACTCATCGAG
      ACGGTCACAA TGTTGGTTAA TTGGTTAAGA CTAATCTTTT TGAGTAGCTC
.....
5901  CATCAAATGA AACTGCAATT TATTCATATC AGGATTATCA ATACCATATT
      GTAGTTTACT TTGACGTTAA ATAAGTATAG TCCTAATAGT TATGGTATAA
.....
5951  TTTGAAAAAG CCGTTTCTGT AATGAAGGAG AAAACTCACC GAGGCAGTTC
      AAACTTTTTC GGCAAAGACA TTACTTCCTC TCTTGAGTGG CTCCGTCAAG
.....
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6001  CATAGGATGG CAAGATCCTG GTATCGGTCT GCGATTCCGA CTCGTCCAAC
      GTATCCTACC GTTCTAGGAC CATAGCCAGA CGCTAAGGCT GAGCAGGTTG

6051  ATCAATACAA CCTATTAATT TCCCCTCGTC AAAAATAAGG TTATCAAGTG
      TAGTTATGTT GGATAATTAA AGGGGAGCAG TTTTTATTCC AATAGTTCAC

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                                                    ~~~~~~~
6101  AGAAATCACC ATGAGTGACG ACTGAATCCG GTGAGAATGG CAAAAGCTTA
      TCTTTAGTGG TACTCACTGC TGACTTAGGC CACTCTTACC GTTTTCGAAT

6151  TGCATTTCTT TCCAGACTTG TTCAACAGGC CAGCCATTAC GCTCGTCATC
      ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG GTCGGTAATG CGAGCAGTAG

6201  AAAATCACTC GCATCAACCA AACCGTTATT CATTCGTGAT TGCGCCTGAG
      TTTTAGTGAG CGTAGTTGGT TTGGCAATAA GTAAGCACTA ACGCGGACTC

                  PvuI
                  ~ ~~~~
6251  CCAGACGAAA TACGCGATCG CTGTTAAAAG GACAATTACA AACAGGAATC
      GCTCTGCTTT ATGCGCTAGC GACAATTTTC CTGTTAATGT TTGTCCTTAG

6301  GAATGCAACC GGCGCAGGAA CACTGCCAGC GCATCAACAA TATTTTCACC
      CTTACGTTGG CCGCGTCCTT GTGACGGTCG CGTAGTTGTT ATAAAAGTGG

6351  TGAATCAGGA TATTCTTCTA ATACCTGGAA TGCTGTTTTC CCGGGGATCG
      ACTTAGTCCT ATAAGAAGAT TATGGACCTT ACGACAAAAG GGCCCCTAGC

6401  CAGTGGTGAG TAACCATGCA TCATCAGGAG TACGGATAAA ATGCTTGATG
      GTCACCACTC ATTGGTACGT AGTAGTCCTC ATGCCTATTT TACGAACTAC

6451  GTCGGAAGAG GCATAAATTC CGTCAGCCAG TTTAGTCTGA CCATCTCATC
      CAGCCTTCTC CGTATTTAAG GCAGTCGGTC AAATCAGACT GGTAGAGTAG

6501  TGTAACATCA TTGGCAACGC TACCTTTGCC ATGTTTCAGA AACAACTCTG
      ACATTGTAGT AACCGTTGCG ATGGAAACGG TACAAAGTCT TTGTTGAGAC

                            ClaI
                            ~~~~~
6551  GCGCATCGGG CTTCCCATAC AATCGATAGA TTGTCGCACC TGATTGCCCG
      CGCGTAGCCC GAAGGGTATG TTAGCTATCT AACAGCGTGG ACTAACGGGC

6601  ACATTATCGC GAGCCCATTT ATACCCATAT AAATCAGCAT CCATGTTGGA
      TGTAATAGCG CTCGGGTAAA TATGGGTATA TTTAGTCGTA GGTACAACCT

                  XhoI
                  ~~~~~
6651  ATTTAATCGC GGCCTCGAGC AAGACGTTTC CCGTTGAATA TGGCTCATAA
      TAAATTAGCG CCGGAGCTCG TTCTGCAAAG GGCAACTTAT ACCGAGTATT

6701  CACCCCTTGT ATTACTGTTT ATGTAAGCAG ACAGTTTTAT TGTTCATGAT
      GTGGGGAACA TAATGACAAA TACATTCGTC TGTCAAAATA ACAAGTACTA

                                                    DraIII
                                                    ~~~~~~
6751  GATATATTTT TATCTTGTGC AATGTAACAT CAGAGATTTT GAGACACAAC
      CTATATAAAA ATAGAACACG TTACATTGTA GTCTCTAAAA CTCTGTGTTG
```

DraIII

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6801  GTGGCTTTCC CCCCCCCCCC ATTATTGAAG CATTTATCAG GGTTATTGTC
      CACCGAAAGG GGGGGGGGGG TAATAACTTC GTAAATAGTC CCAATAACAG
.....
6851  TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
      AGTACTCGCC TATGTATAAA CTTACATAAA TCTTTTTATT TGTTTATCCC
.....
6901  GTTCCGCGCA CATTTCCCCG AAAACTGCCA CCTGACGTCT AAGAAACCAT
      CAAGGCGCGT GTAAAGGGGC TTTTCACGGT GGACTGCAGA TTCTTTGGTA
.....
6951  TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTTTC
      ATAATAGTAC TGTAATTGGA TATTTTTATC CGCATAGTGC TCCGGGAAAG
.....
7001  GTC
      CAG
.....
```

Sequence Listing ID No: 2

pVR 1012-GP(S)

General Description

DNA pVR 1012-GP(S)
Local object
Created: 09/14/98 03:58PM
Last Modification Date: ? (no data)
length: 7073 bp
storage type: Basic
form: Circular
Comments

Restriction Map

BalI: 1 site TGGCCA / ACCGGT

BclI: 1 site TGATCA / ACTAGT

ClaI: 1 site ATCGAT / TAGCTA

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

KasI: 1 site GGCGCC / CCGCGG

KpnI: 1 site GGTACC / CCATGG

NarI: 1 site GGCGCC / CCGCGG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

SalI: 1 site GTCGAC / CAGCTG

XbaI: 1 site TCTAGA / AGATCT

XmnI: 1 site GAANNNNTTC / CTTNNNNAAG

NdeI: 2 sites CATATG / GTATAC

EcoRV: 3 sites GATATC / CTATAG

SphI: 3 sites GCATGC / CGTACG

NcoI: 4 sites CCATGG / GGTACC

BamHI: 6 sites GGATCC / CCTAGG

Functional Map

CDS (4 signals)

CMV IE 5' UT

Start: 886 End: 1129
CMV IE INT
Start: 1130 End: 1840
TbGH
Start: 4090 End: 4642
Kan r
Start: 6138 End: 6760 (Complementary)
Misc_feature (2 signals)
CMV enhancer
Start: 248 End: 885
GP(S)
Start: 1870 End: 4089

Annotations

```
   1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
      AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

  51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
      CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

 101  TCAGGGCCCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
      AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
                                       ------
 151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
      GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

                                                      BalI
                                                     ------
 201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
      GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

 251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
      AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

 301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
      AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

 351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
      TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

 401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
      TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

 451  CCCATTCACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
      GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

 501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
      GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                        NdeI
                       ------
 551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
      CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

 601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
      ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
                                                     ------
 651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
      TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

      NcoI
      ----
 701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
      CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

 751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
      TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA
```

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 801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
      CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
 ..........................................................................

 851  TTGACGCAAA TCGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT AGCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 ..........................................................................

 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
 ..........................................................................

                                                  SacII
                                                  ~~~~~~

 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
 ..........................................................................

1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
 ..........................................................................

                                                  SphI
                                                  ~~~~~~

1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
 ..........................................................................

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
 ..........................................................................

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
 ..........................................................................

1201  TATTCGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
 ..........................................................................

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
 ..........................................................................

1301  ACGGACTCTG TATTTTTACA GGATGGCGTC CCATTCATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
 ..........................................................................

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
 ..........................................................................

1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
 ..........................................................................

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCGG AGCCCTGGTC GCATGGCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGCC TCGGGACCAG CGTACCGAGG
 ..........................................................................

1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
 ..........................................................................

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
 ..........................................................................

1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
 ..........................................................................

1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATCCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TAGGTCCGTC
 ..........................................................................
```

```
1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
.....
1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC
.....
                                                      NcoI
                                                      -----
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA
.....
                              SalI
                              ------
      NcoI                         PmlI   BclI   EcoRV
      --                           ~~~~~~~~~~~~~~~~~~~
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC
.....
                      SphI                            EcoRV
                      --------                        -------
1901  CCGCTCTAGC TAGATGCATG CTCGAGCGGC CGCCAGTGTG ATGGATATCT
      GGCGAGATCG ATCTACGTAC GAGCTCGCCG GCGGTCACAC TACCTATAGA
.....
                                 NcoI
                                 --------
1951  GCAGAATTCT ATCTTCAGGA TCTCGCCATG GAGGGTCTTA GCCTACTCCA
      CGTCTTAAGA TAGAAGTCCT AGAGCGGTAC CTCCCAGAAT CGGATGAGGT
.....
2001  ATTGCCCAGA GATAAATTTC GAAAAAGCTC TTTCTTTGTT TGGGTCATCA
      TAACGGGTCT CTATTTAAAG CTTTTTCGAG AAAGAAACAA ACCCAGTAGT
.....
2051  TCTTATTTCA AAAGGCCTTT TCCATGCCTT TGGGTGTTGT GACCAACAGC
      AGAATAAAGT TTTCCGGAAA AGGTACGGAA ACCCACAACA CTGGTTGTCG
.....
2101  ACTTTAGAAG TAACAGAGAT TGACCAGCTA GTCTGCAAGG ATCATCTTGC
      TGAAATCTTC ATTGTCTCTA ACTGGTCGAT CAGACGTTCC TAGTAGAACG
.....
2151  ATCAACTGAC CAGCTGAAAT CAGTTGGTCT CAACCTCGAG GGGAGCGGAG
      TAGTTGACTG GTCGACTTTA GTCAACCAGA GTTGGAGCTC CCCTCGCCTC
.....
            EcoRV
            -------
2201  TATCTACTGA TATCCCATCT GCGACAAAGC GTTGGGGCTT CAGATCTGGT
      ATAGATGACT ATAGGGTAGA CGCTGTTTCG CAACCCCGAA GTCTAGACCA
.....
2251  GTGCCTCCCC AAGTGGTCAG CTATGAAGCA GGAGAATGGG CTGAAAATTG
      CACGGAGGGG TTCACCAGTC GATACTTCGT CCTCTTACCC GACTTTTAAC
.....
2301  CTACAATCTT GAAATAAAGA AACCGGACGG GAGCGAATGC TTACCCCCAC
      GATGTTAGAA CTTTATTTCT TTGGCCTGCC CTCGCTTACG AATGGGGGTG
.....
2351  CGCCGGATGG TGTCAGAGGC TTTCCAAGGT GCCGCTATGT TCACAAAGCC
      GCGGCCTACC ACAGTCTCCG AAAGGTTCCA CGGCGATACA AGTGTTTCGG
.....
2401  CAAGGAACCG GGCCCTGCCC GGGTGACTAT GCCTTTCACA AGGATGGAGC
      GTTCCTTGGC CCGGGACGGG CCCACTGATA CGGAAAGTGT TCCTACCTCG
.....
2451  TTCCTTCCTC TATGACAGGC TGGCCTCAAC TGTAATTTAC AGAGGAGTCA
      AAAGAAGGAG ATACTGTCCG ACCGAAGTTG ACATTAAATG TCTCCTCAGT
.....
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2501  ATTTTGCTGA GGGGGTAATC GCATTCTTGA TATTGGCTAA ACCAAAGGAA
      TAAAACGACT CCCCCATTAG CGTAAGAACT ATAACCGATT TGGTTTCCTT
.....
2551  ACGTTCCTTC AATCACCCCC CATTCGAGAG GCAGCAAACT ACACTGAAAA
      TGCAAGGAAG TTAGTGGGGG GTAAGCTCTC CGTCGTTTGA TGTGACTTTT
.....
2601  TACATCAAGT TACTATGCCA CATCCTACTT GGAGTACGAA ATCGAAAATT
      ATGTAGTTCA ATGATACGGT GTAGGATGAA CCTCATGCTT TAGCTTTTAA
.....
2651  TTGGTGCTCA ACACTCCACG ACCCTTTTCA AAATTAACAA TAATACTTTT
      AACCACGAGT TGTGAGGTGC TGGGAAAAGT TTTAATTGTT ATTATGAAAA
.....
2701  GTTCTTCTGG ACAGGCCCCA CACGCCTCAG TTCCTTTTCC AGCTGAATGA
      CAAGAAGACC TGTCCGGGGT GTGCGGAGTC AAGGAAAAGG TCGACTTACT
.....
2751  TACCATTCAA CTTCACCAAC AGTTGAGCAA CACAACTGGG AAACTAATTT
      ATGGTAAGTT GAAGTGGTTG TCAACTCGTT GTGTTGACCC TTTGATTAAA
.....
2801  GGACACTAGA TGCTAATATC AATGCTGATA TTGGTGAATG GGCTTTTTGG
      CCTGTGATCT ACGATTATAG TTACGACTAT AACCACTTAC CCGAAAAACC
.....
2851  GAAAATAAAA AAATCTCTCC GAACAACTAC GTGGAGAAGA GCTGTCTTTC
      CTTTTATTTT TTTAGAGAGG CTTGTTGATG CACCTCTTCT CGACAGAAAG
.....
2901  GAAACTTTAT CGCTCAACGA GACAGAAGAC CATGATGCCA CATCGTCGAG
      CTTTGAAATA GCGAGTTGCT CTGTCTTCTG CTACTACGCT GTAGCAGCTC
.....
2951  AACTACAAAG GGAAGAATCT CCGACCGGGC CACCAGGAAG TATTCGGACC
      TTGATGTTTC CCTTCTTAGA GGCTGGCCCG GTGGTCCTTC ATAAGCCTGG
.....
3001  TGGTTCCAAA GGATTCCCCT GGGATGGTTT CATTGCACGT ACCAGAAGGG
      ACCAAGGTTT CCTAAGGGGA CCCTACCAAA GTAACGTGCA TGGTCTTCCC
.....
3051  GAAACAACAT TGCTGTCTCA GAATTCGACA GAAGGTCGAA GAGTAGATGT
      CTTTGTTGTA ACCGCAGAGT CTTAAGCTGT CTTCCAGCTT CTCATCTACA
.....
3101  GAATACTCAG GAAACTATCA CAGAGACAAC TGCAACAATC ATAGGCACTA
      CTTATGAGTC CTTTGATAGT GTCTCTGTTG ACGTTGTTAG TATCCGTGAT
.....
3151  ACGGTAACAA CATGCAGATC TCCAGCATCG GGACAGGACT GAGCTGCAGC
      TGCCATTGTT GTACGTCTAG AGGTCGTAGC CCTGTCCTGA CTCGAGGTCG
.....
                          NcoI
3201  CAAATCCTGA GTACGTCACC GACCATGGCA CCAAGCCCTG AGACTCAGAC
      GTTTAGGACT CATGCAGTGG CTGGTACCGT GGTTCGGGAC TCTGAGTCTG
.....
3251  CTCCACAACC TACACACCAA AACTACCAGT GATGACCACC GAGGAACCAA
      GAGGTGTTGG ATGTGTGGTT TTGATGGTCA CTACTGGTGG CTCCTTGGTT
.....
3301  CAACACCACC GAGAAACTCT CCTGGCTCAA CAACAGAAGC ACGCACTCTC
      GTTGTGGTGG CTCTTTGAGA GGACCGAGTT GTTGTCTTCG TGGGTGAGAG
.....
3351  ACCACCCCAG AGAATATAAC AACAGCGGTT AAAACTGTTT GGGCACAAGA
      TGGTGGGGTC TCTTATATTG TTGTCGCCAA TTTTGACAAA CCCGTGTTCT
.....
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3401  GTCCACAAGC AACGGTCTAA TAACTTCAAC AGTAACAGGT ATTCTTGGGA
      CAGGTGTTCG TTGCCAGATT ATTGAAGTTG TCATTGTCCA TAAGAACCCT
.....

3451  GCCTTGGACT TCGAAAACGC AGCAGAAGAC AAGTTAACAC CAGGGCCACG
      CGGAACCTGA AGCTTTTGCG TCGTCTTCTG TTCAATTGTG GTCCCGGTGC
.....

3501  GGTAAATGCA ATCCCAACTT ACACTACTGG ACTGCACAAG AACAACATAA
      CCATTTACGT TAGGGTTGAA TGTGATGACC TGACGTGTTC TTGTTGTATT
.....

                        BamHI
                        ~~~~~~
3551  TGCTGCTGGG ATTGCCTGGA TCCCGTACTT TGCACCGGGT GCAGAAGGCA
      ACGACGACCC TAACGGACCT AGGGCATGAA ACCTGGCCCA CGTCTTCCGT
.....

3601  TATACACTGA AGGCCTTATG CACAACCAAA ATGCCTTAGT CTGTGGACTC
      ATATGTGACT TCCGGAATAC GTGTTGGTTT TACGGAATCA GACACCTGAG
.....

3651  AGACAACTTG CAAATGAAAC AACTCAAGCT CTGCAGCTTT TCTTAAGGGC
      TCTGTTGAAC GTTTACTTTG TTGAGTTCGA GACGTCGAAA AGAATTCCCG
.....

3701  CACGACGGAG CTGCGGACAT ATACCATACT CAATAGGAAG GCCATAGATT
      GTGCTGCCTC GACGCCTGTA TATGGTATGA GTTATCCTTC CGGTATCTAA
.....

                                    BamHI
                                    ~~~~~~
3751  TCCTTCTGCG ACGATGGGGC GGGACATGTA GGATCCTGGG ACCAGATTGT
      AGGAAGACGC TGCTACCCCG CCCTGTACAT CCTAGGACCC TGGTCTAACA
.....

3801  TGCATTGAGC CACATCATTG GACCAAAAAC ATCACTGATA AAATCAACCA
      ACGTAACTCG GTGTACTAAC CTGGTTTTTG TAGTGACTAT TTTAGTTGGT
.....

3851  AATCATCCAT GATTTCATCG ACAACCCTTT ACCCAATCAG GATAATGATG
      TTAGTAGGTA CTAAAGTAGC TGTTGGGAAA TGGGTTAGTC CTATTACTAC
.....

                                  BamHI
                                  ~~~~~~
3901  ATAATTGGTG GACGGGCTGG AGACAGTGGA TCCCTGCAGG AATAGGCATT
      TATTAACCAC CTGCCCGACC TCTGTCACCT AGGGACGTCC TTATCCGTAA
.....

3951  ACTGGAATTA TTATTGCAAT CATTGCTCTT CTTTGCGTCT GCAAGCTGCT
      TGACCTTAAT AATAACGTTA GTAACGAGAA GAAACGCAGA CGTTCGACGA
.....

                                                    BamHI
                                                    ~~~~~~
4001  TTGTGGAATA TCAGAATTCC AGCACTGGCG GCCGTTACTA GTGGATCCGA
      AACACCTTAT AGTCTTAAGG TCGTGACCGC CGGCAATGAT CACCTAGGCT
.....

                                NarI
                                ~~~~~~
        BamHI       XbaI        KasI     BamHI
        ~~~~~~      ~~~~~~      ~~~~~~   ~~~~~~
4051  GCTCGGATCC AAGCTCTAGA CCAGGCGCCT GGATCCAGAT CTGCTGTGCC
      CGAGCCTAGG TTCGAGATCT GGTCCGCGGA CCTAGGTCTA GACGACACGG
.....

4101  TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA
      AAGATCAACG GTCGGTAGAC AACAAACGGG GAGGGGGCAC GGAAGGAACT
.....

4151  CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT
      GGGACCTTCC ACGGTGAGGG TGACAGGAAA GGATTATTTT ACTCCTTTAA
.....
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```
4201  GCATCGCATT CTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG
      CGTAGCGTAA CAGACTCATC CACAGTAAGA TAAGACCCCC CACCCCACCC
.....

                                                  SphI
                                                  ------
4251  GCAGCACAGC AAGGCGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG
      CGTCGTGTCG TTCCCCCTCC TAACCCTTCT GTTATCGTCC GTACGACCCC
.....

                        KpnI
                        ----
4301  ATGCGGTGGG CTCTATGGGT ACCCAGGTGC TGAAGAATTC ACCCGGTTCC
      TACGCCACCC GAGATACCCA TGGGTCCACG ACTTCTTAAG TGGGCCAAGG
.....

4351  TCCTGGGCCA GAAAGAAGCA GGCACATCCC CTTCTCTGTG ACACACCCTG
      AGGACCCGGT CTTTCTTCGT CCGTGTAGGG GAAGAGACAC TGTGTGGGAC
.....

4401  TCCACGCCCC TGGTTCTTAG TTCCAGCCCC ACTCATAGGA CACTCATAGC
      AGGTGCGGGG ACCAAGAATC AAGGTCGGGG TGAGTATCCT GTGAGTATCG
.....

4451  TCAGGAGGGC TCCGCCTTCA ATCCCACCCG CTAAAGTACT TGGAGCGGTC
      AGTCCTCCCG AGGCGGAAGT TAGGGTGGGC GATTTCATGA ACCTCGCCAG
.....

4501  TCTCCCTCCC TCATCAGCCC ACCAAACCAA ACCTAGGCTC CAAGAGTGGG
      AGAGGGAGGG AGTAGTCGGG TGGTTTGGTT TGGATCGGAG GTTCTCACCC
.....

4551  AAGAAATTAA AGCAAGATAG GCTATTAAGT GCAGAGGGAG AGAAAATGCC
      TTCTTTAATT TCGTTCTATC CGATAATTCA CGTCTCCCTC TCTTTTACGG
.....

                                          XmnI
                                          ----
4601  TCCAACATGT GAGGAAGTAA TGAGAGAAAT CATAGAATTT CTTCCGCTTC
      AGGTTGTACA CTCCTTCATT ACTCTCTTTA GTATCTTAAA GAAGGCGAAG
.....

4651  CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
      GAGCGAGTGA CTGAGCGACG CGAGCCAGCA AGCCGACGCC GCTCGCCATA
.....

4701  CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC
      GTCGAGTGAG TTTCCGCCAT TATGCCAATA GGTGTCTTAG TCCCCTATTG
.....

4751  GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
      CGTCCTTTCT TGTACACTCG TTTTCCGGTC GTTTTCCGGT CCTTGGCATT
.....

4801  AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
      TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG GGACTGCTCG
.....

4851  ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
      TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT
.....

4901  TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT
      ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG CGAGAGGACA
.....

4951  TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
      AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG GGAAGCCCTT
.....

5001  GCGTGGCGCT TTCTCAATGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG
      CGCACCGCGA AAGAGTTACG AGTGCGACAT CCATAGAGTC AAGCCACATC
.....
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5051  GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA
      CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC AAGTCGGGCT
.....
5101  CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC
      GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG GGCCATTCTG
.....
5151  ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
      TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA ATCGTCTCGC
.....
5201  AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG
      TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG GATTGATGCC
.....
5251  CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
      GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC TTCGGTCAAT
.....
5301  CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT
      GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT TTGGTGGCGA
.....
5351  GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
      CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG CGTCTTTTTT
.....
5401  AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT
      TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA
.....
5451  GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
      CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA TAGTTTTTCC
.....
5501  ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA
      TAGAAGTGGA TCTAGGAAAA TTTAATTTTT ACTTCAAAAT TTAGTTAGAT
.....
5551  AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
      TTCATATATA CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC
.....
5601  AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA
      TCCGTGGATA GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT
.....
5651  CTCCGGGGGG GGGGGGCGCT GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG
      GAGGCCCCCC CCCCCCGCGA CTCCAGACGG AGCACTTCTT CCACAACGAC
.....
5701  ACTCATACCA GGCCTGAATC GCCCCATCAT CCAGCCAGAA AGTGAGGGAG
      TGAGTATGGT CCGGACTTAG CGGGGTAGTA GGTCGGTCTT TCACTCCCTC
.....
5751  CCACGGTTGA TGAGAGCTTT GTTGTAGGTG GACCAGTTGG TGATTTTGAA
      GGTGCCAACT ACTCTCGAAA CAACATCCAC CTGGTCAACC ACTAAAACTT
.....
5801  CTTTTGCTTT GCCACGGAAC GGTCTGCGTT GTCGGGAAGA TGCGTGATCT
      GAAAACGAAA CGGTGCCTTG CCAGACGCAA CAGCCCTTCT ACGCACTAGA
.....
5851  GATCCTTCAA CTCAGCAAAA GTTCGATTTA TTCAACAAAG CCGCCGTCCC
      CTAGGAAGTT GAGTCGTTTT CAAGCTAAAT AAGTTGTTTC GGCGGCAGGG
.....
5901  GTCAAGTCAG CGTAATGCTC TGCCAGTGTT ACAACCAATT AACCAATTCT
      CAGTTCAGTC GCATTACGAG ACGGTCACAA TGTTGGTTAA TTGGTTAAGA
.....
5951  GATTAGAAAA ACTCATCGAG CATCAAATGA AACTGCAATT TATTCATATC
      CTAATCTTTT TGAGTAGCTC GTAGTTTACT TTGACGTTAA ATAAGTATAG
.....
```

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6001  AGGATTATCA ATACCATATT TTTGAAAAAG CCGTTTCTGT AATGAAGGAG
      TCCTAATAGT TATGGTATAA AAACTTTTTC GGCAAAGACA TTACTTCCTC
.....
6051  AAAACTCACC GAGGCAGTTC CATAGGATGG CAAGATGCTG GTATCGGTCT
      TTTTGAGTGG CTCCGTCAAG GTATCCTACC GTTCTAGGAC CATAGCCAGA
.....
6101  GCGATTCCGA CTCGTCCAAC ATCAATACAA CCTATTAATT TCCCCTGGTC
      CGCTAAGGCT GAGCAGGTTG TAGTTATGTT GGATAATTAA AGGGGACCAG
.....
6151  AAAAATAAGG TTATCAAGTG AGAAATCACC ATGAGTGACG ACTGAATCCG
      TTTTTATTCC AATAGTTCAC TCTTTAGTGG TACTCACTGC TGACTTAGGC
.....
                  HindIII
                  ========
6201  GTGAGAATGG CAAAAGCTTA TGCATTTCTT TCCAGACTTG TTCAACAGGC
      CACTCTTACC GTTTTCGAAT ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG
.....
6251  CAGCCATTAC GCTCGTCATC AAAATCACTC GCATCAACCA AACCGTTATT
      GTCGGTAATG CGAGCAGTAG TTTTAGTGAG CGTAGTTGGT TTGGCAATAA
.....
                                        PvuI
                                        ====
6301  CATTCGTGAT TGCGCCTGAG CGAGACGAAA TACGCGATCG CTGTTAAAAG
      GTAAGCACTA ACGCGGACTC GCTCTGCTTT ATGCGCTAGC GACAATTTTC
.....
6351  GACAATTACA AACAGGAATC GAATGCAACC GGCGCAGGAA CACTGCCAGC
      CTGTTAATGT TTGTCCTTAG CTTACGTTGG CCGCGTCCTT GTGACGGTCG
.....
6401  GCATCAACAA TATTTTCACC TGAATCAGGA TATTCTTCTA ATACCTGGAA
      CGTAGTTGTT ATAAAAGTGG ACTTAGTCCT ATAAGAAGAT TATGGACCTT
.....
6451  TGCTGTTTTC CCGGGGATCG CAGTGGTGAG TAACCATGCA TCATCAGGAG
      ACGACAAAAG GGCCCCTAGC GTCACCACTC ATTGGTACGT AGTAGTCCTC
.....
6501  TACGGATAAA ATGCTTGATG GTCGGAAGAG GCATAAATTC CGTCAGCCAG
      ATGCCTATTT TACGAACTAC CAGCCTTCTC CGTATTTAAG GCAGTCGGTC
.....
6551  TTTAGTCTGA CCATCTCATC TGTAACATCA TTGGCAACGC TACCTTTGCC
      AAATCAGACT GGTAGAGTAG ACATTGTAGT AACCGTTGCG ATGGAAACGG
.....
                                                  ClaI
                                                  ====
6601  ATGTTTCAGA AACAACTCTG GCGCATCGGG CTTCCCATAC AATCGATAGA
      TACAAAGTCT TTGTTGAGAC CGCGTAGCCC GAAGGGTATG TTAGCTATCT
.....
6651  TTGTCGCACC TGATTGCCCG ACATTATCGC GAGCCCATTT ATACCCATAT
      AACAGCGTGG ACTAACGGGC TGTAATAGCG CTCGGGTAAA TATGGGTATA
.....
6701  AAATCAGCAT CCATGTTGGA ATTTAATCGC GGCCTCGAGC AAGACGTTTC
      TTTAGTCGTA GGTACAACCT TAAATTAGCG CCGGAGCTCG TTCTGCAAAG
.....
6751  CCGTTGAATA TGGCTCATAA CACCGCTTGT ATTACTGTTT ATGTAAGCAG
      GGCAACTTAT ACCGAGTATT GTGGGGAACA TAATGACAAA TACATTCGTC
.....
6801  ACAGTTTTAT TGTTCATGAT GATATATTTT TATCTTGTGC AATGTAACAT
      TGTCAAAATA ACAAGTACTA CTATATAAAA ATAGAACACG TTACATTGTA
.....
```

DraIII

```
6851  CAGAGATTTT GAGACACAAC GTGGCTTTCC CCCCCCCCCC ATTATTGAAG
      GTCTCTAAAA CTCTGTGTTG CACCGAAAGG GGGGGGGGGG TAATAACTTC
.....
6901  CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT
      GTAAATAGTC CCAATAACAG AGTACTCGCC TATGTATAAA CTTACATAAA
.....
6951  AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA
      TCTTTTTATT TGTTTATCCC CAAGGCGCGT GTAAAGGGGC TTTTCACGGT
.....
7001  CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG
      GGACTGCAGA TTCTTTGGTA ATAATAGTAC TGTAATTGGA TATTTTTATC
.....
7051  GCGTATCACG AGGCCCTTTC GTC
      CGCATAGTGC TCCGGGAAAG CAG
.....
```

Sequence Listing ID No: 3

pVR 1012-GP(Z)

General Description

DNA pVR 1012-GP(Z)
Local object
Created: 09/15/98 05:06PM
Last Modification Date: ? (no data)
length: 7285 bp
storage type: Basic
form: Circular

Comments

Restriction Map

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KasI: 1 site GGCGCC / CCGCGG

NarI: 1 site GGCGCC / CCGCGG

NotI: 1 site GCGGCCGC / CGCCGGCG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

XbaI: 1 site TCTAGA / AGATCT

XhoI: 1 site CTCGAG / GAGCTC

EcoRV: 2 sites GATATC / CTATAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1860

TbGH
Start: 4303 End: 4854

Kan r
Start: 6350 End: 6972 (Complementary)

Misc_feature (2 signals)

CMV enhancer

Start: 248 End: 885

GP(Z)

Start: 1870 End: 4301

Annotations

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  1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTGGCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT CCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                    NdeI
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGCGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 ....................................................................................

 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
 ....................................................................................

                                                 SacII
                                                 ------

 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT
 ....................................................................................

1001  CCGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
 ....................................................................................

                                                 SphI
                                                 ----

1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
 ....................................................................................

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
 ....................................................................................

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
 ....................................................................................

1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
 ....................................................................................

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
 ....................................................................................

1301  ACGGACTCTC TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAG ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
 ....................................................................................

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
 ....................................................................................

1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
 ....................................................................................

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
 ....................................................................................

1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
 ....................................................................................

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
 ....................................................................................

1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
 ....................................................................................

1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
 ....................................................................................

1701  CTGAGTTCTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
 ....................................................................................
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        HpaI
1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

                                                        NcoI
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

      NcoI                              PmlI          EcoRVNotI
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

                    NarI
      NotI XbaI     KasI
1901  CCGCTCTAGA CCAGGCGCCT GGATCGATCC GCGATGAAGA TTAAGCCGAC
      GGCGAGATCT GGTCCGCGGA CCTAGCTAGG CGCTACTTCT AATTCGGCTG

1951  AGTGAGCGTA ATCTTCATCT CTCTTAGATT ATTTGTTTTC CAGAGTAGGG
      TCACTCGCAT TAGAAGTAGA GAGAATCTAA TAAACAAAAG GTCTCATCCC

2001  GTCGTCAGGT CCTTTTCAAT CGTGTAACCA AAATAAACTC CACTAGAAGG
      CAGCAGTCCA GGAAAAGTTA GCACATTGGT TTTATTTGAG GTGATCTTCC

2051  ATATTGTGGG GCAACAACAC AATGGGCGTT ACAGGAATAT TGCAGTTACC
      TATAACACCC CGTTGTTGTG TTACCCGCAA TGTCCTTATA ACGTCAATGG

2101  TCGTGATCGA TTCAAGAGGA CATCATTCTT TCTTTGGGTA ATTATGCTTT
      AGCACTAGCT AAGTTCTCCT GTAGTAAGAA AGAAACCCAT TAATAGGAAA

2151  TCCAAAGAAC ATGTTCCATC CCACTTGGAG TCATCCACAA TAGCACATTA
      AGGTTTCTTG TAAAAGGTAG GGTGAACCTC AGTAGGTGTT ATCGTGTAAT

2201  CAGGTTAGTG ATGTCGACAA ACTAGTTTGT CGTGACAAAC TGTCATCCAC
      GTCCAATCAC TACAGCTGTT TGATCAAACA GCACTGTTTG ACAGTAGGTG

2251  AAATCAATTG AGATCAGTTG GACTGAATCT CGAAGCGAAT GGAGTGGCAA
      TTTAGTTAAC TCTAGTCAAC CTGACTTAGA GCTTCGCTTA CCTCACCGTT

2301  CTGACGTGCC ATCTGCAACT AAAAGATGGG GCTTCAGGTC CGGTGTCCCA
      GACTGCACGG TAGACGTTGA TTTTCTACCC CGAAGTCCAG GCCACAGGGT

2351  CCAAAGGTGG TCAATTATGA AGCTGGTGAA TGGGCTGAAA ACTGCTACAA
      GGTTTCCACC AGTTAATACT TCGACCACTT ACCCGACTTT TGACGATGTT

2401  TCTTGAAATC AAAAAACCTG ACGGCAGTGA GTGTCTACCA GCAGCGCCAG
      AGAACTTTAG TTTTTTGGAC TGCCGTCACT CACAGATGGT CGTCGCGGTC

2451  ACGGGATTCG GGGCTTCCCC CGGTGCCCGT ATGTGCACAA AGTATCAGGA
      TGCCCTAAGC CCCGAAGGGG GCCACGGGCA TACACGTGTT TCATAGTCCT

2501  ACGGGACCGT GTGCCGGAGA CTTTGCCTTC CATAAAGAGG GTGCTTCTTT
      TGCCCTGGCA CACGGCCTCT GAAACGGAAG GTATTTCTCC CACGAAAGAA
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2551  CCTGTATGAT CGACTTGCTT CCACAGTTAT CTACCGAGGA ACGACTTTCG
      GGACATACTA GCTGAACGAA GGTGTCAATA GATGGCTCCT TGCTGAAAGC
2601  CTGAAGGTGT CGTTGCATTT CTGATACTGC CCCAAGCTAA GAAGGACTTC
      GACTTCCACA GCAACGTAAA GACTATGACG GGGTTCGATT CTTCCTGAAG
2651  TTCAGCTCAC ACCCCTTGAG AGAGCCGGTC AATGCAACGG AGGACCCGTC
      AAGTCGAGTG TGGGGAACTC TCTCGGCCAG TTACGTTGCC TCCTGGGCAG
                                    EcoRV
                                   ------
2701  TAGTGGCTAC TATTCTACCA CAATTAGATA TCAGGCTACC GGTTTTGGAA
      ATCACCGATG ATAAGATGGT GTTAATCTAT AGTCCGATGG CCAAAACCTT
2751  CCAATGAGAC AGAGTACTTG TTCGAGGTTG ACAATTTGAC CTACGTCCAA
      GGTTACTCTG TCTCATGAAC AAGCTCCAAC TGTTAAACTG GATGCAGGTT
2801  CTTGAATCAA GATTCACACC ACAGTTTCTG CTCCAGCTGA ATGAGACAAT
      GAACTTAGTT CTAAGTGTGG TGTCAAAGAC GAGGTCGACT TACTCTGTTA
2851  ATATACAAGT GGGAAAAGGA GCAATACCAC GGCAAAACTA ATTTGGAAGG
      TATATGTTCA CCCTTTTCCT CGTTATGGTG CCGTTTTGAT TAAACCTTCC
2901  TCAACCCCGA AATTGATACA ACAATCGGGG AGTGGGCCTT CTGGGAAACT
      AGTTGGGGCT TTAACTATGT TGTTAGCCCC TCACCCGGAA GACCCTTTGA
2951  AAAAAAAACC TCACTAGAAA AATTCGCAGT GAAGAGTTGT CTTTCACAGT
      TTTTTTTTGG AGTGATCTTT TTAAGCGTCA CTTCTCAACA GAAAGTGTCA
3001  TGTATCAAAC GGAGCCAAAA ACATCAGTGG TCAGAGTCCG GCGCGAACTT
      ACATAGTTTG CCTCGGTTTT TGTAGTCACC AGTCTCAGGC CGCGCTTGAA
3051  CTTCCGACCC AGGGACCAAC ACAACAACTG AAGACCACAA AATCATGGCT
      GAAGGCTGGG TCCCTGGTTG TGTTGTTGAC TTCTGGTGTT TTAGTACCGA
3101  TCAGAAAATT CCTCTGCAAT GGTTCAAGTG CACAGTCAAG GAAGGGAAGC
      AGTCTTTTAA GGAGACGTTA CCAAGTTCAC GTGTCAGTTC CTTCCCTTCG
3151  TGCAGTGTCG CATCTAACAA CCCTTGCCAC AATCTCCACG AGTCCCCAAT
      ACGTCACAGC GTAGATTGTT GGGAACGGTG TTAGAGGTGC TCAGGGGTTA
3201  CCCTCACAAC CAAACCAGGT CCGGACAACA GCACCCATAA TACACCCGTG
      GGGAGTGTTG GTTTGGTCCA GGCCTGTTGT CGTGGGTATT ATGTGGGCAC
3251  TATAAACTTG ACATCTCTGA GGCAACTCAA GTTGAACAAC ATCACCGCAG
      ATATTTGAAC TGTAGAGACT CCGTTGAGTT CAACTTGTTG TAGTGGCGTC
3301  AACAGACAAC GACAGCACAG CCTCCGACAC TCCCTCTGCC ACGACCGCAG
      TTGTCTGTTG CTGTCGTGTC GGAGGCTGTG AGGGAGACGG TGCTGGCGTC
3351  CCGGACCCCC AAAAGCAGAG AACACCAACA CGAGCAAGAG CACTGACTTC
      GGCCTGGGGG TTTTCGTCTC TTGTGGTTGT GCTCGTTCTC GTGACTGAAG
3401  CTGGACCCCG CCACCACAAC AAGTCCCCAA AACCACAGCG AGACCGCTGG
      GACCTGGGGC GGTGGTGTTG TTCAGGGGTT TTGGTGTCGC TCTGGCGACC
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3451  CAACAACAAC ACTCATCACC AAGATACCGG AGAAGAGAGT GCCAGCAGCG
      GTTGTTGTTG TGAGTAGTGG TTCTATGGCC TCTTCTCTCA CGGTCGTCGC
.....
3501  GGAAGCTAGG CTTAATTACC AATACTATTG CTGGAGTCGC ACGACTGATC
      CCTTCGATCC GAATTAATGG TTATGATAAC GACCTCAGCG TCCTGACTAG
.....
3551  ACAGGCGGGA GAAGAACTCG AAGAGAAGCA ATTGTCAATG CTCAAGCCAA
      TGTCCGCCCT CTTCTTGAGC TTCTCTTCGT TAACAGTTAC GAGTTGGGTT
.....
3601  ATGCAACCCT AATTTACATT ACTGGACTAC TCAGGATGAA GGTGCTGCAA
      TACGTTGGGA TTAAATGTAA TGACCTGATG AGTCCTACTT CCACGACGTT
.....
3651  TCGGACTGGC CTGGATACCA TATTTCGGGC CAGCAGCCGA GGCAATTTAC
      AGCCTGACCG GACCTATGGT ATAAAGCCCG GTCGTCGGCT CCGTTAAATG
.....
3701  ATAGAGGGGC TAATGCACAA TCAAGATGGT TTAATCTGTG GGTTGAGACA
      TATCTCCCCG ATTACGTGTT AGTTCTACCA AATTAGACAC CCAACTCTGT
.....
3751  GCTGGCCAAC GAGACGACTC AAGCTCTTCA ACTGTTCCTG AGAGCCACAA
      CGACCGGTTG CTCTGCTGAG TTCGAGAAGT TGACAAGGAC TCTCGGTGTT
.....
3801  CTGAGCTACG CACCTTTTCA ATCCTCAACC GTAAGGCAAT TGATTCCTTG
      GACTCGATGC GTGGAAAAGT TAGGAGTTGG CATTCCGTTA ACTAAAGAAC
.....
3851  CTGCAGCGAT GGGGCGGCAC ATGCCACATT CTGGGACCGG ACTGCTGTAT
      GACGTCGCTA CCCCGCCGTG TACGGTGTAA GACCCTGGCC TGACGACATA
.....
3901  CGAACCACAT GATTGGACCA AGAACATAAC AGACAAAATT GATCAGATTA
      GCTTGGTGTA CTAACCTGGT TCTTGTATTG TCTGTTTTAA CTAGTCTAAT
.....
3951  TTCATGATTT TGTTGATAAA ACCCTTCCCG ACCAGGGGGA CAATGACAAT
      AAGTACTAAA ACAACTATTT TGGGAAGGCC TGGTCCCCCT GTTACTGTTA
.....
4001  TGGTGGACAG GATGGAGACA ATGGATACCG GCAGGTATTG GAGTTACAGG
      ACCACCTGTC CTACCTCTGT TACCTATGGC CGTCCATAAC CTCAATGTCC
.....
4051  CGTTATAATT GCAGTTATCG CTTTATTCTG TATATGCAAA TTTGTCTTTT
      GCAATATTAA CGTCAATAGC GAAATAAGAC ATATACGTTT AAACAGAAAA
.....
4101  AGATTTTCTT CAGATTGCTT CATGGAAAAG CTCAGCCTCA AATCAATGAA
      TCAAAAAGAA GTCTAACGAA GTACCTTTTC GAGTCGGAGT TTAGTTACTT
.....
4151  ACCAGGATTT AATTATATGG ATTACTTGAA TCTAAGATTA CTTGACAAAT
      TGGTCCTAAA TTAATATACC TAATGAACTT AGATTCTAAT GAACTGTTTA
.....
4201  GATAATATAA TACACTGGAG CTTTAAACAT AGCCAATGTG ATTCTAACTC
      CTATTATATT ATGTGACCTC GAAATTTGTA TCGGTTACAC TAAGATTGAG
.....
4251  CTTTAAACTC ACAGTTAATC ATAAACAAGG TTTGGTACCG AGCTGGAATT
      GAAATTTGAG TGTCAATTAG TATTTGTTCC AAACCATGGC TCGAGCTTAA
.....
4301  ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTGCCGCG
      TAGACGACAC GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGCGC
.....
4351  TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA
      ACGGAAGGAA CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT
.....
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4401  AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG
      TTACTCCTTT AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC
.....
4451  GGGTGGGGTG GGGCAGCACA GCAAGGGGGA CGATTGGGAA GACAATAGCA
      CCCACCCCAC CCCGTCGTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT
.....
        SphI
       ------
4501  GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCCAGGT GCTGAAGAAT
      CCGTACGACC CCTACGCCAC CCGAGATACC CATGGGTCCA CGACTTCTTA
.....
4551  TGACCCGGTT CCTCCTGGGC CAGAAAGAAG CAGGCACATC CCCTTCTCTG
      ACTGGGCCAA GGAGGACCCG GTCTTTCTTC GTCCGTGTAG GGGAAGAGAC
.....
4601  TGACACACCC TGTCCACGCC CCTGGTTCTT AGTTCCAGCC CCACTCATAG
      ACTGTGTGGG ACAGGTGCGG GGACCAAGAA TCAAGGTCGG GGTGAGTATC
.....
4651  GACACTCATA GCTCAGGAGG GCTCCGCCTT CAATCCCACC CGCTAAAGTA
      CTGTGAGTAT CGAGTCCTCC CGAGGCGGAA GTTAGGGTGG GCGATTTCAT
.....
4701  CTTGGAGCGG TCTCTCCCTC CCTCATCAGC CCACCAAACC AAACCTAGCC
      GAACCTCGCC AGAGAGGGAG GGAGTAGTCG GGTGGTTTGG TTTGGATCGG
.....
4751  TCCAAGAGTG GGAAGAAATT AAAGCAAGAT AGGCTATTAA GTGCAGAGGG
      AGGTTCTCAC CCTTCTTTAA TTTCGTTCTA TCCGATAATT CACGTCTCCC
.....
4801  AGAGAAAATG CCTCCAACAT GTGAGGAAGT AATGAGAGAA ATCATAGAAT
      TCTCTTTTAC GGAGGTTGTA CACTCCTTCA TTACTCTCTT TAGTATCTTA
.....
4851  TTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
      AAGAAGGCGA AGGAGCGAGT GACTGAGCGA CGCGAGCCAG CAAGCCGACG
.....
4901  GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
      CCGCTCGCCA TAGTCGAGTG AGTTTCCGCC ATTATGCCAA TAGGTGTCTT
.....
4951  TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
      AGTCCCCTAT TGCGTCCTTT CTTGTACACT CGTTTTCCGG TCGTTTTCCG
.....
5001  CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC
      GTCCTTGGCA TTTTTCCGGC GCAACGACCG CAAAAAGGTA TCCGAGGCGG
.....
5051  CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
      GGGGACTGCT CGTAGTGTTT TTAGCTGCGA GTTCAGTCTC CACCGCTTTG
.....
5101  CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT
      GGCTGTCCTG ATATTTCTAT GGTCCGCAAA GGGGGACCTT CGAGGGAGCA
.....
5151  GCGCTCTGCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC
      CGCGAGAGGA CAAGGCTGGG ACGGCGAATG GCCTATGGAC AGGCGGAAAG
.....
5201  TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG TAGGTATCTC
      AGGGAAGCCC TTCGCACCGC GAAAGAGTTA CGAGTGCGAC ATCCATAGAG
.....
5251  AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
      TCAAGCCACA TCCAGCAAGC GAGGTTCGAC CCGACACACG TGCTTGGGGG
.....
```

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5301  CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA
      GCAAGTCGGG CTGGCGACGC GGAATAGGCC ATTGATAGCA GAACTCAGGT
.....
5351  ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG
      TGGGCCATTC TGTGCTGAAT AGCGGTGACC GTCGTCGGTG ACCATTGTCC
.....
5401  ATTAGCAGAG CGAGGTATGT AGCCGGTGCT ACAGAGTTCT TGAAGTGGTG
      TAATCGTCTC GCTCCATACA TCCGCCACGA TGTCTCAAGA ACTTCACCAC
.....
5451  GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC
      CGGATTGATG CCGATGTGAT CTTCCTGTCA TAAACCATAG ACGCGAGACG
.....
5501  TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
      ACTTCGGTCA ATGGAAGCCT TTTTCTCAAC CATCGAGAAC TAGGCCGTTT
.....
5551  CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
      GTTTGGTGGC GACCATCGCC ACCAAAAAAA CAAACGTTCG TCGTCTAATG
.....
5601  GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT
      CGCGTCTTTT TTTCCTAGAG TTCTTCTAGG AAACTAGAAA AGATGCCGCA
.....
5651  CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA
      GACTGCGAGT CACCTTGCTT TTGAGTGCAA TTCCCTAAAA CCAGTACTCT
.....
5701  TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT
      AATAGTTTTT CCTAGAAGTG GATCTAGGAA AATTTAATTT TTACTTCAAA
.....
5751  TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
      ATTTAGTTAG ATTTCATATA TACTCATTTG AACCAGACTG TCAATGGTTA
.....
5801  GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
      CGAATTAGTC ACTCCGTGGA TAGAGTCGCT AGACAGATAA AGCAAGTAGG
.....
5851  ATAGTTGCCT GACTCCGGGG GGGGGGGGCG CTGAGGTCTG CCTCGTGAAG
      TATCAACGGA CTGAGGCCCC CCCCCCCCGC GACTCCAGAC GGAGCACTTC
.....
5901  AAGGTGTTGC TGACTCATAC CAGGCCTGAA TCGCCCCATC ATCCAGCCAG
      TTCCACAACG ACTGAGTATG GTCCGGACTT AGCGGGGTAG TAGGTCGGTC
.....
5951  AAAGTGAGGG AGCCACGGTT GATGAGAGCT TTGTTGTAGG TGGACCAGTT
      TTTCACTCCC TCGGTGCCAA CTACTCTCGA AACAACATCC ACCTGGTCAA
.....
6001  GGTGATTTTG AACTTTTGCT TTGCCACGGA ACGGTCTGCG TTGTCGGGAA
      CCACTAAAAC TTGAAAACGA AACGGTGCCT TGCCAGACGC AACAGCCCTT
.....
6051  GATGCGTGAT CTGATCCTTC AACTCAGCAA AAGTTGGATT TATTCAACAA
      CTACGCACTA GACTAGGAAG TTGAGTCGTT TTCAAGCTAA ATAAGTTGTT
.....
6101  AGCCGCCGTC CCGTCAAGTC AGCGTAATGC TCTGCCAGTG TTACAACCAA
      TCGGCGGCAG GGCAGTTCAG TCGCATTACG AGACGGTCAC AATGTTGGTT
.....
6151  TTAACCAATT CTGATTAGAA AAACTCATCG AGCATCAAAT GAAACTGCAA
      AATTGGTTAA GACTAATCTT TTTGAGTAGC TCGTAGTTTA CTTTGACGTT
.....
6201  TTTATTCATA TCAGGATTAT CAATACCATA TTTTTGAAAA AGGCGTTTCT
      AAATAAGTAT AGTCCTAATA GTTATGGTAT AAAAACTTTT TCGGCAAAGA
.....
```

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6251  GTAATGAAGG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC
      CATTACTTCC TCTTTTGAGT GGCTCCGTCA AGGTATCCTA CCGTTCTAGG
.....
6301  TGGTATCGGT CTGCGATTCC GACTCGTCCA ACATCAATAC AACCTATTAA
      ACCATAGCCA GACGCTAAGG CTGAGCAGGT TGTAGTTATG TTGGATAATT
.....
6351  TTTCCCCTCG TCAAAAATAA GGTTATCAAG TGAGAAATCA CCATGAGTGA
      AAAGGGGAGC AGTTTTTATT CCAATAGTTC ACTCTTTAGT GGTACTCACT
.....
                                HindIII
                                ~~~~~~~
6401  CGACTGAATC CGGTGAGAAT GGCAAAAGCT TATGCATTTC TTTCCAGACT
      GCTGACTTAG GCCACTCTTA CCGTTTTCGA ATACGTAAAG AAAGGTCTGA
.....
6451  TGTTCAACAG GCCAGCCATT ACGCTCGTCA TCAAAATCAC TCGCATCAAC
      ACAAGTTGTC CGGTCGGTAA TGCGAGCAGT AGTTTTAGTG AGCGTAGTTG
.....
                                                      PvuI
                                                      ~~~~
6501  CAAACCGTTA TTCATTCGTG ATTGCGCCTG AGCGAGACGA AATACGCGAT
      GTTTGGCAAT AAGTAAGCAC TAACGCGGAC TCGCTCTGCT TTATGCGCTA
.....
      PvuI
      ~~
6551  CGCTGTTAAA AGGACAATTA CAAACAGGAA TCGAATGCAA CCGGCGCAGG
      GCGACAATTT TCCTGTTAAT GTTTGTCCTT AGCTTACGTT GGCCGCGTCC
.....
6601  AACACTGCCA GCGCATCAAC AATATTTTCA CCTGAATCAG GATATTCTTC
      TTGTGACGGT CGCGTAGTTG TTATAAAAGT GGACTTAGTC CTATAAGAAG
.....
6651  TAATACCTGG AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG
      ATTATGGACC TTACGACAAA AGGGCCCCTA GCGTCACCAC TCATTGGTAC
.....
6701  CATCATCAGG AGTACGGATA AAATGCTTGA TGGTCGGAAG AGGCATAAAT
      GTAGTAGTCC TCATGCCTAT TTTACGAACT ACCAGCCTTC TCCGTATTTA
.....
6751  TCCGTCAGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC
      AGGCAGTCGG TCAAATCAGA CTGGTAGAGT AGACATTGTA GTAACCGTTG
.....
6801  GCTACCTTTG CCATGTTTCA GAAACAACTC TGGCGCATCG GGCTTCCCAT
      CGATGGAAAC GGTACAAAGT CTTTGTTGAG ACCGCGTAGC CCGAAGGGTA
.....
6851  ACAATCGATA GATTGTCGCA CCTGATTGCC CGACATTATC GCGAGCCCAT
      TGTTAGCTAT CTAACAGCGT GGACTAACGG GCTGTAATAG CGCTCGGGTA
.....
                                                      XhoI
                                                      ~~~~~
6901  TTATACCCAT ATAAATCAGC ATCCATGTTG GAATTTAATC GCGGCCTCGA
      AATATGGGTA TATTTAGTCG TAGGTACAAC CTTAAATTAG CGCCGGAGCT
.....
      XhoI
      ~
6951  GCAAGACGTT TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT
      CGTTCTGCAA AGGGCAACTT ATACCGAGTA TTGTGGGGAA CATAATGACA
.....
7001  TTATGTAAGC AGACAGTTTT ATTGTTCATG ATGATATATT TTTATCTTGT
      AATACATTCG TCTGTCAAAA TAACAAGTAC TACTATATAA AAATAGAACA
.....
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DraIII

```
7051  GCAATGTAAC ATCAGAGATT TTGAGACACA ACGTGGCTTT CCCCCCCCCC
      CGTTACATTG TAGTCTCTAA AACTCTGTGT TGCACCGAAA GGGGGGGGGG
.....
7101  CCATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT
      GGTAATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA
.....
7151  TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC
      AACTTACATA AATCTTTTTA TTTGTTTATC CCCAAGGCGC GTGTAAAGGG
.....
7201  CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC
      GCTTTTCACG GTGGACTGCA GATTCTTTGG TAATAATAGT ACTGTAATTG
.....
7251  CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTC
      GATATTTTTA TCCGCATAGT GCTCCGGGAA AGCAG
.....
```

Sequence Listing ID NO: 4

pVR 1012-SGP(Z)

General D scription

DNA pVR 1012-SGP(Z)
Local object
Created: 09/14/98 04:29PM
Last Modified: 09/15/98 04:50PM
length: 7272 bp
storage type: Basic
form: Circular
Comments

Restriction Map

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

HpaI: 1 site GTTAAC / CAATTG

KpnI: 1 site GGTACC / CCATGG

NotI: 1 site GCGGCCGC / CGCCGGCG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

XbaI: 1 site TCTAGA / AGATCT

XhoI: 1 site CTCGAG / GAGCTC

EcoRV: 2 sites GATATC / CTATAG

NcoI: 2 sites CCATGG / GGTACC

NdeI: 2 sites CATATG / GTATAC

SphI: 2 sites GCATGC / CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1840

TbGH
Start: 4289 End: 4861

Kan r
Start: 6337 End: 6959 (Complementary)

Misc_feature (2 signals)

CMV enhancer
Start: 248 End: 885
SGP(Z)
Start: 1870 End: 4288

Annotations

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  1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
                                       ------
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                      NdeI
                     ------
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
                                                     ------
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
     -
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGCCGGGGT
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 851  TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC
 ....................................................................................

 901  AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA
 ....................................................................................

                                                 SacII
                                                 ------

 951  TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCGGGCCCTT
 ....................................................................................

1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG
 ....................................................................................

                                                 SphI
                                                 ------

1051  CCATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA
 ....................................................................................

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC
 ....................................................................................

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG
 ....................................................................................

1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT
 ....................................................................................

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG
 ....................................................................................

1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA
 ....................................................................................

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT
 ....................................................................................

1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA
 ....................................................................................

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG
 ....................................................................................

1501  AGCGGCTCAT CGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC
 ....................................................................................

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC
 ....................................................................................

1601  TCGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      AGCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC
 ....................................................................................

1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
 ....................................................................................

1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
 ....................................................................................
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        HpaI
        ~~~~~
1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC
.....

                                                        NcoI
                                                        ~~~~~
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA
.....

      NcoI                              PmlI          EcoRVNotI
      ~~                                ~~~~~~~       ~~~~~~~~~~
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC
.....

      NotI XbaI
      ~~~~~~~~~~
1901  CCGCTCTAGA CCAGGCGCCT GGATCGAATT GATGAAGATT AAGCCGACAG
      GGCGAGATCT GGTCCGCGGA CCTAGCTTAA CTACTTCTAA TTCGGCTGTC
.....

1951  TGAGCGTAAT CTTCATCTCT CTTAGATTAT TTGTTTTCCA GAGTAGGGGT
      ACTCGCATTA GAAGTAGAGA GAATCTAATA AACAAAAGGT CTCATCCCCA
.....

2001  CGTCAGGTCC TTTTCAATCG TGTAACCAAA ATAAACTCCA CTAGAAGGAT
      GCAGTCCAGG AAAAGTTAGC ACATTGGTTT TATTTGAGGT GATCTTCCTA
.....

2051  ATTGTGGGGC AACAACACAA TGGGCGTTAC AGGAATATTG CAGTTACCTC
      TAACACCCCG TTGTTGTGTT ACCCGCAATG TCCTTATAAC GTCAATGGAG
.....

2101  GTGATCGATT CAAGAGGACA TCATTCTTTC TTTGGGTAAT TATCCTTTTC
      CACTAGCTAA GTTCTCCTGT AGTAAGAAAG AAACCCATTA ATAGGAAAAG
.....

2151  CAAAGAACAT TTTCCATCCC ACTTGGAGTC ATCCACAATA GCACATTACA
      GTTTCTTGTA AAAGGTAGGG TGAACCTCAG TAGGTGTTAT CGTGTAATGT
.....

2201  GGTTAGTGAT GTCGACAAAC TAGTTTGTCG TGACAAACTG TCATCCACAA
      CCAATCACTA CAGCTGTTTG ATCAAACAGC ACTGTTTGAC AGTAGGTGTT
.....

2251  ATCAATTGAG ATCAGTTGGA CTGAATCTCG AAGGGAATGG AGTGGCAACT
      TAGTTAACTC TAGTCAACCT GACTTAGAGC TTCCCTTACC TCACCGTTGA
.....

2301  GACGTGCCAT CTGCAACTAA AAGATGGGGC TTCAGGTCCG GTGTCCCACC
      CTGCACGGTA GACGTTGATT TTCTACCCCG AAGTCCAGGC CACAGGGTGG
.....

2351  AAAGGTGGTC AATTATGAAG CTGGTGAATG GGCTGAAAAC TGCTACAATC
      TTTCCACCAG TTAATACTTC GACCACTTAC CCGACTTTTG ACGATGTTAG
.....

2401  TTGAAATCAA AAAACCTGAC GGGAGTGAGT GTCTACCAGC AGCGCCAGAC
      AACTTTAGTT TTTTGGACTG CCCTCACTCA CAGATGGTCG TCGCGGTCTG
.....

2451  GCGATTCGGG GCTTCCCCCG GTGCCGGTAT GTGCACAAAG TATCAGGAAC
      CGCTAAGCCC CGAAGGGGGC CACGGCCATA CACGTGTTTC ATAGTCCTTG
.....

2501  GGGACCGTGT GCCGGAGACT TTGCCTTCCA TAAAGAGGGT GCTTTCTTCC
      CCCTGGCACA CGGCCTCTGA AACGGAAGGT ATTTCTCCCA CGAAAGAAGG
.....

2551  TGTATGATCG ACTTGCTTCC ACAGTTATCT ACCGAGGAAC GACTTTCGCT
      ACATACTAGC TGAACGAAGG TGTCAATAGA TGGCTCCTTG CTGAAAGCGA
.....
```

```
2601  GAAGGTGTCG TTGCATTTCT GATACTGCCC CAAGCTAAGA AGGACTTCTT
      CTTCCACAGC AACGTAAAGA CTATGACGGG GTTCGATTCT TCCTGAAGAA
.....
2651  CAGCTCACAC CCCTTGAGAG AGCCGGTCAA TGCAACGGAG GACCCGTCTA
      GTCGAGTGTG GGGAACTCTC TCGGCCAGTT ACGTTGCCTC CTGGGCAGAT
.....
                                  EcoRV
                                  ------
2701  GTGGCTACTA TTCTACCACA ATTAGATATC AGGCTACCGG TTTTGGAACC
      CACCGATGAT AAGATGGTGT TAATCTATAG TCCGATGGCC AAAACCTTGG
.....
2751  AATGAGACAG AGTACTTCTT CGAGGTTGAC AATTTGACCT ACGTCCAACT
      TTACTCTGTC TCATGAACAA GCTCCAACTG TTAAACTGGA TGCAGGTTGA
.....
2801  TGAATCAAGA TTCACACCAC AGTTTCTGCT CCAGCTGAAT GAGACAATAT
      ACTTAGTTCT AAGTGTGGTG TCAAAGACGA GGTCGACTTA CTCTGTTATA
.....
2851  ATACAAGTGG GAAAAGGAGC AATACCACGG GAAAACTAAT TTGGAAGGTC
      TATGTTCACC CTTTTCCTCG TTATGGTGCC CTTTTGATTA AACCTTCCAG
.....
2901  AACCCCGAAA TTGATACAAC AATCGGGGAG TGGGCCTTCT GGGAAACTAA
      TTGGGGCTTT AACTATGTTG TTAGCCCCTC ACCCGGAAGA CCCTTTGATT
.....
2951  AAAAACCTCA CTAGAAAAAT TCGCAGTGAA GAGTTGTCTT TCACAGTTGT
      TTTTTGGAGT GATCTTTTTA AGCGTCACTT CTCAACAGAA AGTGTCAACA
.....
3001  ATCAAACGGA GCCAAAAACA TCAGTGGTCA GAGTCCGGCG CGAACTTCTT
      TAGTTTGCCT CGGTTTTTGT AGTCACCAGT CTCAGGCCGC GCTTGAAGAA
.....
3051  CCGACCCAGG GACCAACACA ACAACTGAAG ACCACAAAAT CATGGCTTCA
      GGCTGGGTCC CTGGTTGTGT TGTTGACTTC TGGTGTTTTA GTACCGAAGT
.....
3101  GAAAATTCCT CTGCAATGGT TCAAGTGCAC AGTCAAGGAA GGGAAGCTGC
      CTTTTAAGGA GACGTTACCA AGTTCACGTG TCAGTTCCTT CCCTTCGACG
.....
3151  AGTGTCGCAT CTAACAACCC TTGCCACAAT CTCCACGAGT GCCCAATCCC
      TCACAGCGTA GATTGTTGGG AACGGTGTTA GAGGTGCTCA GGGGTTAGGG
.....
3201  TCACAACCAA ACCAGGTCCG GACAACAGCA CCCATAATAC ACCCGTGTAT
      AGTGTTGGTT TGGTCCAGGC CTGTTGTCGT GGGTATTATG TGGGCACATA
.....
3251  AAACTTGACA TCTCTGAGGC AACTCAAGTT GAACAACATC ACCGCAGAAC
      TTTGAACTGT AGAGACTCCG TTGAGTTCAA CTTGTTGTAG TGGCGTCTTG
.....
3301  AGACAACGAC AGCACAGCCT CCGACACTCC CTCTGCCACG ACCGCAGCCG
      TCTGTTGCTG TCGTGTCGGA GGCTGTGAGG GAGACGGTGC TGGCGTCGGC
.....
3351  GACCCCCAAA AGCAGAGAAC ACCAACACGA GCAAGAGCAC TGACTTCCTG
      CTGGGGGTTT TCGTCTCTTG TGGTTGTGCT CGTTCTCGTG ACTGAAGGAC
.....
3401  GACCCCGCCA CCACAACAAG TGCCCAAAAC CACAGCGAGA CCGCTGGCAA
      CTGGGGCGGT GGTGTTGTTC AGGGGTTTTG GTGTCGCTCT GGCGACCGTT
.....
3451  CAACAACACT CATCACCAAG ATACCGGAGA AGAGAGTGCC AGCAGCGGGA
      GTTGTTGTGA GTAGTGGTTC TATGGCCTCT TCTCTCACGG TCGTCGCCCT
.....
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3501  AGCTAGGCTT AATTACCAAT ACTATTGCTG GAGTCGCAGG ACTGATCACA
      TCGATCCGAA TTAATGGTTA TGATAACGAC CTCAGCGTCC TGACTAGTGT
.....
3551  GGCGGGAGAA GAACTCGAAG AGAAGCAATT GTCAATGCTC AACCCAAATG
      CCGCCCTCTT CTTGAGCTTC TCTTCGTTAA CAGTTACGAG TTGGGTTTAC
.....
3601  CAACCCTAAT TTACATTACT GGACTACTCA GGATGAAGGT GCTGCAATCG
      GTTGGGATTA AATGTAATGA CCTGATGAGT CCTACTTCCA CGACGTTAGC
.....
3651  GACTGGCCTG GATACCATAT TTCGGGCCAG CAGCCGAGGG AATTTACATA
      CTGACCGGAC CTATGGTATA AAGCCCGGTC GTCGGCTCCC TTAAATGTAT
.....
3701  GAGGGGCTAA TGCACAATCA AGATGGTTTA ATCTGTGGGT TGAGACAGCT
      CTCCCCGATT ACGTGTTAGT TCTACCAAAT TAGACACCCA ACTCTGTCGA
.....
3751  GGCCAACGAG ACGACTCAAG CTCTTCAACT GTTCCTGAGA GCCACAACTG
      CCGGTTGCTC TGCTGAGTTC GAGAAGTTGA CAAGGACTCT CGGTGTTGAC
.....
3801  AGCTACGCAC CTTTTCAATC CTCAACCGTA AGGCAATTGA TTTCTTGCTG
      TCGATGCGTG GAAAAGTTAG GAGTTGGCAT TCCGTTAACT AAAGAACGAC
.....
3851  CAGCGATGGG GCGGCACATG CCACATTCTG GGACCGGACT GCTGTATCGA
      GTCGCTACCC CGCCGTGTAC GGTGTAAGAC CCTGGCCTGA CGACATAGCT
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3951  ATGATTTTGT TGATAAAACC CTTCCGGACC AGGGGGACAA TGACAATTGG
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4301  TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC
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4351  CCTGGAAGGT GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG
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4451 CAGCACAGCA AGGGGGAGGA TTGGGAAGAC AATAGCAGGC ATGCTGGGGA
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KpnI

4501 TGCGGTGGGC TCTATGGGTA CCCAGGTGCT GAAGAATTGA CCCGGTTCCT
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4551 CCTGGGCCAG AAAGAAGCAG GCACATCCCC TTCTCTGTGA CACACCCTGT
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4601 CCACCCCCCT GGTTCTTAGT TCCAGCCCCA CTCATAGGAC ACTCATAGCT
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4651 CAGGAGGGCT CCGCCTTCAA TCCCACCCGC TAAAGTACTT GGAGCGGTCT
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4701 CTCCCTCCCT CATCAGCCCA CCAAACCAAA CCTAGCCTCC AAGAGTGGGA
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4751 AGAAATTAAA GCAAGATAGG CTATTAAGTC CAGAGGGAGA GAAAATGCCT
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5251 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC
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7001  CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC
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7101  ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA
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7151  GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC
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7251  CGTATCACGA GGCCCTTTCG TC
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27364

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/12, 45/00, 39/145, 39/155, 39/205
US CL :424/199.1, 204.1, 209.1, 211.1, 224.1, 278.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/199.1, 204.1, 209.1, 211.1, 224.1, 278.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PALESE, P. et al. Negative-Strand RNA Viruses: Genetic Engineering and Applications Proc. Natl. Acad. Sci. USA October 1996, Vol. 93, pages 11354-11358, see entire document	1-26
Y	SANCHEZ, A. et al. The Virion Glycoproteins of Ebola Viruses are Encoded in Two Reading Frames and are Expressed Through Transcriptional Editing Proc. Natl. Acad. Sci. USA. April 1996, Vol. 93, pages 3602-3607, see entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on, or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 APRIL 1999	Date of mailing of the international search report 10 MAY 1999
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